# STUDIES ON NON-SYMBIOTIC NITROGEN FIXATION IN SOME ALBERTA SOILS

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# "STUDIES ON NON-SYMBIOTIC NITROGEN FIXATION IN SOME ALBERTA SOILS"

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## INTRODUCTION

Since one of the elements of protein is nitrogen, all living things on this planet, whether animal or vegetable, must have nitrogen in their food. This great demand for nitrogen means that large quantities of nitrogen are removed every year from the land.

The earth's atmosphere contains enough nitrogen to replenish this annual nitrogen loss, yet not nearly enough of this free nitrogen is converted by artificial synthesis into combined nitrogen. Therefore man depends to a large extent upon nature to capture and fix atmospheric nitrogen.

One of nature's methods of helping man is to provide microorganisms that are capable of assimilating atmospheric nitrogen. These microorganisms belong to two major groups, usually designated as non-symbiotic and symbiotic nitrogen-fixing bacteria.

The symbiotic nitrogen-fixing organisms, as the name indicates, fix nitrogen while living symbiotically with leguminous plants. This relationship has been known since 1886, and the value of leguminous crops to mankind has been known for thousands of years.

In 1893 Winogradsky isolated anaerobic non-symbiotic nitrogen fixing bacteria which he called Clostridium pasteurianum. Eight years later Beijerinck isolated aerobic forms to which he gave the generic name Azotobacter. Following the discovery of these nitrogen-fixing organisms which could lead a free existence within the soil, keen interest was taken in their study.

Milne (60) investigated non-symbiotic nitrogen fixation in Western Canadian soils. However Milne's (60) experimental work was mostly confined to Saskatchewan soils. Also no information was obtained as to whether the nitrogen fixing organisms from these Canadian soils could fix nitrogen under apparently practical conditions. Thus, besides determining the relative nitrogen fixing powers of various Alberta soils, it was felt that additional work should be done to secure data regarding the effect, on non-symbiotic nitrogen fixation, of some of the cultural practices used by Alberta farmers.

For these reasons some attention was paid to the following problems: (1) the application of straw to soil culture to find out if the byproducts of decomposing straw could be utilized as energy for non-symbiotic nitrogen fixation, (2) the effects of cultivation on the soils' nitrogen-fixing powers, (3) in southern Alberta the application of irrigation waters frequently leave the soil in a water-logged condition. Therefore some work was done to determine the effect that waterlogging has upon non-symbiotic nitrogen fixation, (4) also attempts were made to determine the effect

of fertilization upon non-symbiotic nitrogen fixation.

# REVIEW OF LITERATURE

Since the discovery in 1891 by Winogradsky, as reviewed by Waksman (75), of an anaerobic organism belonging to the group of butyric acid bacteria and capable of fixing atmospheric nitrogen non-symbiotically, a voluminous literature on non-symbiotic nitrogen fixation has accumulated. Therefore, because of such an abundance of available literature it is only possible in this thesis to review the more pertinent aspects and fundamentals of non-symbiotic nitrogen fixation.

It is proposed here to divide the literature review into two parts, namely, Part I and Part II. Part I, the major portion of the literature review, will cover non-symbiotic nitrogen fixation. Part II, the minor portion,will give an extremely brief review of the decomposition of plant residues. The inclusion of this latter part was deemed desirable because a considerable amount of the author's work pertains to the ascertainment of the beneficial action that decomposing straw has upon non-symbiotic nitrogen fixation.

## Part I: Non-symbiotic Nitrogen Fixation

Stephenson (71) remarks, "the faculty of using molecular nitrogen for the synthesis of cell material is, so far as we know, confined to micro-organisms, and is not shared by any of the more complex forms of life."

Waksman and Starkey (78) report that these micro-

organisms can be divided into two groups: (1) the non-symbiotic bacteria which lead a free existence in the soil and obtain energy from various compounds found in the soil, (2) the symbiotic nitrogen-fixing bacteria which live in symbiosis with certain plant roots, namely the legumeroots.

(1) Non-symbiotic Nitrogen-Fixing Organisms

These free existing organisms are generally divided into two groups; the aerobic organisms and the anaerobic organisms.

(a) Aerobic Organisms

This group is largely represented by the genus Azotobacter.

Bergey's Manual of Determinative Bacteriology (6) lists three species; Az. chroococcum, Az. agilis and Az. indicum. Az. chroococcum Beijerinck, the type species, is listed as a rod, 2.0 to 3.0 by 3.0 to 6.0 microns, occuring in pairs and packets and occasionally in chains. The cells show three or four refractile granules. The organisms are surrounded by a slimy membrane of variable thickness, usually becoming brownish in older cultures, due possibly to the conversion of tyrosine to melanin. They are gram-negative and motile by means of numerous peritrichious flagella. On gelatin the colonies are very small, circular, yellow, granular, later becoming yellowish-brown. This organism produces only slight growth even in the presence of glucose, and peptone is utilized with difficulty. Litmus milk is reduced in 10 to 14 days. On potato, colonies are glossy, barely visible, slimy to wrinkled and may become

yellowish, brownish-yellow or chocolate brown. The organism fixes atmospheric nitrogen and gives off carbon dioxide, utilizing such carbon compounds as fructose, maltose, glucose, sucrose, mannitol, inulin, dextrin, galactose, arabinose, starch, glycerol, ethyl alcohol, acetate, butyrate, citrate, lactate, malate, propionate and succinate. It has an optimum temperature of 25°C. to 28°C., and occurs naturally in the majority of neutral or alkaline soils.

Waksman (76) reports that some soil microbiologists recognize only two or three species. However, he states that there are five known species. Besides the three mentioned by Bergey (6) he claims there are also Az. beijerinckii and Az. vinelandii.

Earlier workers such as Bonazzi (7) and Jones (41) found peculiar morphological phases occuring in azotobacter cultures. Jones (40) claimed that azotobacter had heat-resistant endospores and that the visible granules were reproductive organs.

Lohnis and Smith (55) claimed that not less than seven different morphological types can be developed and stabilized from azotobacter cultures.

Later workers like Eisenstark et al (15), with the aid of an electron microscope found that azotobacter had four distinct morphological cell types: (1) one day old cultures were predominately large plump rods, (2) after two or three days of growth cells became somewhat smaller, (3) two and three week old cultures showed long branching rods and thread forms, (4) cells from cultures one month old were predominately minute

coccoid cells.

Eisenstark et al (16) found balloon forms and other morphologically abnormal cells if azotobacter were grown on nutrient agar or soil extract agar, but couldn't obtain these peculiar shapes if grown on nitrogen-free medium.

Eisenstark and McMahon (14) using phase microscopy observed that azotobacter cells had a clear zone surrounding internal dark areas. On other bacteria this clear zone represents the polysaccharide capsule or slime layer. However, in the case of these azotobacter cells this clear area would not take a capsule stain. Consequently the authors concluded that perhaps azotobacter cells are differentiated into true cytoplasmic areas and true nuclear areas as are the cells of higher plants.

(b) Anaerobic Organisms

Waksman and Starkey (78) claim that the anaerobic nitrogen-fixing species are generally referred to as Clostridium pasteurianum but the anaerobic forms embrace numerous closely related forms most of which produce butyric acid when utilizing carbohydrate material.

Bergey (6) mentions that both Cl. pasteurianum and Cl. butyricum fix atmospheric nitrogen and have many other similar characteristics.

According to Bergey (6) Clostridium pasteurianum is a motile rod 0.7 by 5.0 to 7.0 microns, straight or slightly curved, with rounded ends, occuring singly, in pairs and in short chains. Their spores are oval, excentric to subterminal, swelling rods to clostridal forms. These organisms are gram-positive becoming gram-negative and do not liquefy gelatin. On

plain agar slant (anaerobic), little or no growth occurs. On glucose agar (anaerobic), surface colonies are circular, slightly raised, moist and creamy white; the subsurface colonies are biconvex, dense, yellowish-white, entire and the agar is fragmented early by abundant gas. Blood agar is not hemolyzed. In plain broth there is little or no growth. In glucose broth, there is abundant, diffuse turbidity with much gas produced. In litmus milk, the organism produces acid and early coagulates the milk with stormy fermentation. It produces acid and gas from glucose, sucrose, inulin, galactose, fructose, and dextrin. Glycerol, starch, lactose and mannitol are not fermented. This organism is distinguished from Clostridium butyricum by the non-fermentation of starch, and it fixes atmospheric nitrogen and grows well at 30°C. to 37°C.

(c) Other Non-symbiotic Nitrogen-Fixing Organisms

As reviewed by Allison et al (3) Drewes found that certain blue-green algae, especially species of Anabaena and Nostoc had the ability to fix atmospheric nitrogen when grown on nitrogen-free medium.

Allison et al (3) extensively investigated the nitrogen-fixing powers of Nostoc muscorum and found that the organism readily obtains its nitrogen and carbon from the atmosphere. The quantity of nitrogen fixed was as high as 10 milligrams nitrogen and 18 milligrams of nitrogen in 45 days and 85 days respectively per 100 ml.of carbohydrate free medium.

In recent years some other photosynthetic organisms have been found to have the ability to fix atmospheric nitrogen.

The first experimenters to report on this subject were Kamen and Gest (44). They used isotopic nitrogen ($N^{15}$) and Rhodospirillium rubrum, a member of the family Athiorhodaceae, and found that the organism could fix atmospheric nitrogen when cultured both in the dark and in the sunlight.

Wall et al (80) used three members of the family Athiorhodaceae and found them to be able to fix atmospheric nitrogen.

Lindstrom et al (51) found five members of the family Athiorhodaceae and nine different strains of rhodopseudomonads were able to fix atmospheric nitrogen.

(2) Mechanism of Nitrogen Fixation

The mechanism of nitrogen fixation, by organisms, has attracted the attention of many workers. The conclusions made from their experimentations resulted in the formulating of many hypotheses which were and are still speculative. Hence it is proposed here to review only the two recent hypotheses which appear to be the most reasonable.

(a) The Hydroxylamine Hypothesis

As reviewed by Wilson and Burris (82) Virtanen and Laine found that in inoculated leguminous plants: (1) aspartic acid is the sole nitrogenous compound excreted in any quantity, (2) extremely small quantities of oximinosuccinic acid are detected in the excretory products, (3) oxalacetic acid is found in the plants, (4) excised nodules fix more nitrogen when oxalacetic acid is supplied.

Waksman (76) states that Virtanen found aspartic acid in young cultures of azotobacter before ammonia could be detected. Wilson (8) states that Endres found hydroxylamine in azotobacter cultures and since the hydroxylamine was found in the presence, but not in the absence, of free nitrogen Endres concluded that it originated from nitrogen fixation. Wilson (82) also reports that Virtanen found that about five percent of the nitrogen fixed by azotobacter is excreted as aspartic acid with traces of hydroxylamine.

Waksman (76) states that Virtanen suggested the following series of reactions:

$$N_2 \xrightarrow{2H} HN{=}{=}NH \xrightarrow{H_2O} NH_2OH + C_6H_{12}O_6 \longrightarrow HOOC\cdot CO\cdot CH_2\cdot COOH$$

Hydroxylamine — Oxalacetic acid

$$HOOC\cdot C(NOH)\cdot CH_2\cdot COOH \longrightarrow HOOC\cdot CH(NH_2)CH_2\cdot COOH$$

Oxime — Aspartic acid

$$HOOC\cdot CH(NH_2)CH_2\cdot COOH + H_3C\cdot CO\cdot COOH \longrightarrow HOOC\cdot CO\cdot CH_2\cdot COOH + CH_3\cdot CH(NH_2)\cdot COOH$$

Aspartic acid — Pyruvic acid — Oxalacetic acid — Alanine

(copied from Waksman (76))

(b) The Ammonia Hypothesis

As reviewed by Burk and Horner (9) Kostytchev, working with azotobacter, found measureable quantities of ammonia in a culture medium separated from the cells and pellicles. Winogradsky, as reviewed by Burk and Horner (9), found the greatest yield of ammonia occurred when the medium was at a pH of 9.0. Consequently Winogradsky claimed that ammonia was the

intermediate product in the fixation of atmospheric nitrogen. The high alkalinity prevents the cells from assimilating the nitrogen as ammonia, hence it accumulated at pH 9.0.

Burk and Horner (9) conducted several experiments and concluded that the presence of ammonia was caused by spontaneous cell decomposition.

Phelps and Wilson (63) reported the occurrence of hydrogenase, the enzyme which activates molecular hydrogen, in azotobacter and rhizobium. Wilson et al (81) also found hydrogenase in azotobacter cells. Lee et al (47) showed evidence that this hydrogenase is capable of transferring molecular hydrogen to methylene blue or molecular oxygen.

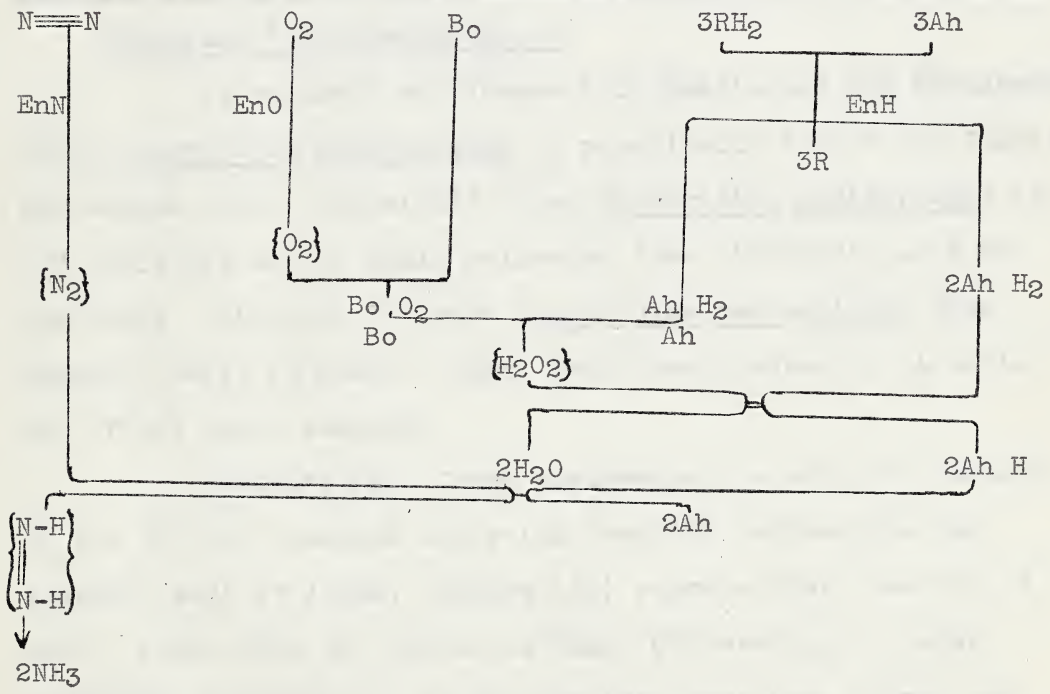
Lee and Wilson (48) found that sources of combined nitrogen which readily inhibit nitrogen fixation by azotobacter also inhibit the formation of hydrogenase. Hence, they concluded that hydrogenase is closely related to the nitrogen-fixing system in azotobacter.

Wilson and Burris (83) supply evidence that points to ammonia as the key intermediate in nitrogen fixation by azotobacter. They cite three conclusive results: (1) isotopic nitrogen ($N^{15}$) supplied as nitrogen or ammonia always accumulated in about the same proportions in the cell amino-acid fractions. In glutamic and aspartic acid $N^{15}$ is high; in histidine and arginine it is low, (2) ammonia is immediately accepted as a source of nitrogen even if the organisms have been previously grown in the presence of atmospheric nitrogen, (3) the organisms will only assimilate combined nitrogen, at a rate comparable to

free nitrogen, from compounds which are readily converted to ammonia.

Wall et al (80) found that photosynthetic bacteria could utilize ammonia as readily as nitrogen and the nitrogen always accumulated in about the same proportions in the cell amino-acid fractions. Rosenblum and Wilson (65) and (66) report that ammonia can be utilized by Clostridium pasteurianum.

Wilson and Burris (83) in support of the ammonia hypothesis proposed the following scheme.




EnN - nitrogen activating enzyme

EnO - oxygen " "

EnH - hydrogen " "

Ah - hydrogen acceptor

Bo - oxygen "

$RH_2$ - substrate

{ } - activated molecules

(copied from Wilson and Burris (83)).

From the foregoing papers it appears that these two hypotheses (which are the latest) are conflicting. The most impressive array of experimental data has been gathered for the ammonia hypothesis. Therefore, it would seem that ammonia is the most likely intermediate product in nitrogen fixation. However, as Wilson and Burris (83) point out, no one has disproved Burk and Horner's (9) findings that ammonia is the result of catabolic instead of anabolic origin.

(3) Distribution and Numbers of Non-symbiotic Nitrogen-Fixing Organisms In Different Soils

As reviewed by Waksman (74) Omeliansky and Freudenreich found Clostridium pasteurianum in practically all of the Russian and German soils. Haselhoff found Clostridium pasteurianum in 137 soils out of 152 soils collected from different parts of the world. Riccardo isolated Clostridium pasteurianum from Vesuvian soils in Italy. Burri found azotobacter in 34 soils out of 105 soils sampled.

Yamagata (85) found azotobacter in about 35 percent of the 300 soil samples collected from the northern to the southern part of Japan. Ohmasa (62) reports that 8 out of 29 soils, taken from the forest of Tokyo University, in Japan, contained azotobacter. In the Szechuei province, China, Gaw (25) found that azotobacter was present in 78 percent of all soils examined. Altson (4) found azotobacter in certain Malayan soils where the pH was above 6.0

Gainey (24) states that about half of the soils in

Kansas contain azotobacter. The organisms were seldom present in virgin, but were abundant in cultivated soils. Jones and Murdock (42) isolated azotobacter from 9 out of 17 soil types examined, and from 22 out of 29 soil samples representing 9 types in Ontario. Lochead and Thexton (54) working with Ontario soils found: (1) azotobacter were consistently higher in unfertilized soils than in fertilized soils, (2) the numbers of azotobacter showed no relation to the productivity of the soils.

Joffe (39) found that the cultivated soils in Crimea are more active in nitrogen-fixing powers than the virgin soils. Martin (57) reports that 82 out of 94 cultivated soil samples taken from Arizona contained azotobacter, while only 27 out of 119 virgin soil samples contained azotobacter. He also reported that 19.6 milligrams of nitrogen were fixed per gram of cultivated soil and only 5.3 milligrams of nitrogen were fixed per gram of virgin soil.

Vandecaveye and Moodie (73) working with Washington soils observed: (1) the azotobacter population of virgin and cultivated soils in non-irrigated areas were the same, (2) azotobacter were abundant in the irrigated soils of the 10 inch rainfall area, (3) azotobacter were present in the non-irrigated soils of the 15-20 inch rainfall area.

Greaves and Bracken (30) found numbers and fixing powers of the bacteria were twice as high in Utah cultivated soils as in virgin soils. Milne (60) working with Saskatchewan soils found the rate and amount of nitrogen fixed was similar

in sand cultures inoculated with cultivated and virgin soils.

Waksman (76) reports that Duggeli found: (1) 100 - 1,000,000 anaerobic and 0 - 100,000 aerobic nitrogen-fixing bacteria per gram of soil, (2) plots treated with sodium nitrate contained 1,600 - 12,000 cells of Clostridium and 4,900 - 6,300 Azotobacter, per gram of soil, (3) plots treated with potassium and phosphorus contained 98,700 Azotobacter and 1,120,000 Clostridium per gram of soil.

Waksman (76) also states that Rossi found an average of 1,815 Azotobacter cells per gram of Italian soil, and Swaby isolated Azotobacter from 26 percent of all soil samples examined.

Spiegelberg (68) found Cl. pasteurianum as a causitive organism in spoiled canned pineapple.

It is evident from the above that the anaerobic organisms are more widely distributed than the aerobic organisms. This wider distribution is partly due to the anaerobic organisms' ability to tolerate a wider range of pH, which will be discussed later.

Also of interest is the fact that cultivation and irrigation appear to increase the numbers and fixing powers of the aerobic organisms in some cases.

(4) Factors Influencing Non-symbiotic Nitrogen Fixation.

(a) Utilizable Energy Sources

The large number of energy materials used in laboratory cultures has already been mentioned under the heading, "Non-symbiotic Nitrogen-Fixing Organisms." However, apparently

there are sources of energy added by nature and farming practices that are of sufficient importance to be discussed here separately.

Winters (84) reports that manure promotes nitrogen fixation.

Richards (64) showed that azotobacter alone is unable to fix an appreciable amount of nitrogen in feeces, but in combination with B. lactis aerogenes it fixes three times as much nitrogen as when alone.

McBeth (58) added dextrose and cellulose to: (1) a pure culture of azotobacter, (2) a mixed culture of azotobacter and Bacillus rossica (a cellulose decomposing bacterium). He found, after a suitable incubation period, that the mixed culture gave an appreciable increase in nitrogen over the azotobacter culture alone.

Krishna (46), using sandy cultures found that both azotobacter and Cl. pasteurianum could utilize, as energy, the byproducts of straw decomposition.

Hutchinson (37), at Rothamsted, added 5 and 10 percent of wheat straw to soil contained in erlenmeyer flasks and found: (1) the 5 percent level of straw gave a more significant increase in nitrogen fixation than the 10 percent level of straw, (2) the use of sand and $CaCO_3$ in conjunction with the experiment gave the best results. This was probably due to the neutralizing of the acid products formed, (3) the addition of 10 percent of elm leaves stimulated nitrogen fixation to the same extent as 5 percent of wheat straw.

Fulmer (20) added 1.5 percent of green clover, wheat and oats straw to field and garden soils contained in pots. The soil moisture was maintained at 25 percent and the incubation temperature was 25°C. Upon nitrogen analysis after a suitable incubation period he concluded: (1) green manures, as clover, wheat or oats when added to soil favor nitrogen fixation, (2) the non-legumes stimulated fixation more than the legumes.

Jensen (38) showed that if actinomycetes were associated with azotobacter, the latter were unable to utilize the byproducts of cellulose decomposition.

(b) Effect of Aeration

Although Clostridium pasteurianum is an obligate anaerobe, Stephenson (71), in reviewing Winogradsky's work, reports that in the presence of contaminating aerobes it can exist under apparently aerobic conditions. The aerobic organisms consume the oxygen, during growth, and create sufficiently anaerobic conditions to permit neighboring cells of Clostridium to develop. This symbiotic condition may account for nitrogen fixation in soil-sand cultures apparently devoid of azotobacter.

Stephenson (71) states that the physical conditions of a medium which tend to increase surface area and hence improve aeration are beneficial for aerobic nitrogen fixers. Thus agar, sand, gypsum and colloidal silica are beneficial.

Hunter (36) bubbled sterile air through azotobacter liquid cultures and found: (1) aeration gave a prompt and

vigorous growth, (2) aeration increased nitrogen fixation, (3) calcium carbonate is not essential to pure cultures undergoing aeration.

(c) Effect of Temperature

In his earlier work Ashby (6) found: (1) azotobacter are active in arid soils to a depth of four feet, (2) they are confined to the upper few inches in humid soil regions. Temperature is probably one of the factors responsible for this difference.

As reviewed by Greene (33) Heinze found that nitrogen-assimilating organisms were most active between 20° and 30°C. but were still able to fix nitrogen at 8°C. Greene (33) also reports that Koch obtained 3, 11, and 15.6 milligrams of nitrogen fixed per 100 gm. of soil incubated at 7°, 15° and 24° C. respectively.

Greene (op. cit.) using different incubation temperatures compared the nitrogen-fixing powers of the azotobacter flora of Arizona soils, which had been subjected to high temperatures for a long period of time, with azotobacter from soils of more temperate regions. He found: (1) seven cultures of azotobacter isolated from Arizona soils fixed more nitrogen at temperatures ranging from 25°C. - 40°C., than the azotobacter from the more temperate regions, (2) at 18°C. no difference could be found. These data suggested to him (op. cit.) that azotobacter from Arizona soils have become adapted to their environment, and hence can fix more nitrogen at higher temperatures.

(d) Effect of Reaction

Waksman (74) states that the optimum reaction for Cl. pasteurianum is pH 6.9 - 7.3, but they can develop well at pH 5.7. This is probably one of the contributing factors for its wide occurrence, which has already been discussed.

On the other hand azotobacter, as the literature reveals, can very seldom withstand a greater acidity than pH 6.0. Fred and Davenport (18) report that they found the critical acid value for azotobacter to be pH 6.5 and the alkaline value to be pH 8.6. Gainey (22) found that pH 6.0 was the critical acid value. Burk et al (12) found that the nitrogen-fixing ability of azotobacter ceased below pH 6.0. However, when combined nitrogen was present in the medium the organism could survive a pH of 4.0 by using this form of nitrogen. Ohmasa (62) found azotobacter to be present in Japanese soils if the pH was 6.0 or higher, but not within the range of 4.0 - 5.5. Martin (57) was unable to isolate azotobacter from mountain meadow soils of Arizona where the pH range was 4.9 - 6.2 Milne (60), working with Saskatchewan soils, found viable azotobacter in all but two soils. These were acids soils of pH 5.6 and 5.0.

Starkey and De (69) isolated a new species which they named Azotobacter indicum. It was capable of fixing atmospheric nitrogen at reactions ranging from pH 3.0-9.0. Altson (4) reported isolating a few strains of azotobacter from Malayan soils which tolerated reactions as low as pH 3.6. The strains were not active, however, when inoculated into suitable

media.

(e) Effect of Moisture

As reviewed by Lipman and Sharp (52) Krainsky found that nitrogen-fixing flora were able to fix nitrogen when the soil contained less than one-fourth of the optimum moisture content. Lipman and Sharp (52), using a light sandy soil, found that 20 - 24 percent moisture, on air-dry basis, was the optimum moisture for nitrogen-fixation.

Greaves (28) reports that Lipman and Burgess found that soils after being air-dried and kept in stoppered bottles for periods of 5 to 20 years still retained their nitrogen-fixing powers. Greaves (28) also states that Krainsky found soils should be damp but not wet for maximum nitrogen-fixation, and that Traaen gives the optimum moisture as 25 - 30 percent for a loam soil which he had used. Using calcareous loam Greaves (28) found the moisture content for maximum nitrogen-fixation to lie between 15 and 22 percent. He also found that if too large a quantity of water is supplied there is a tendency to depress the total nitrogen fixed.

(f) Effect of Individual Ions

A considerable amount of research has been reported regarding the role played by various elements in nitrogen-fixation by azotobacter. If there is any literature concerning the effect that various elements have upon the growth of Clostridium pasteurianum, it is unknown to the author. Therefore, any reference to the stimulating or retardation effects that different ions have upon nitrogen-fixation deals only with

azotobacter.

The well-known azotobacter (plaque) test for phosphate deficiency readily shows that azotobacter are sensitive to phosphorous deficiencies. Greene (32) cites Staklasa's research, which indicates that 5.0 - 5.7 milligrams of nitrogen are fixed for every gram of phosphorous used. The minimum required was found to be 2.46 milligrams of phosphorous per gram of glucose consumed. Ziemiecka (86) added phosphate to soils low in soluble phosphate and obtained an increase in azotobacter activity. Katznelson (45) showed that phosphate fertilization of soils enhanced the ability of a soil to support azotobacter growth.

Bortels (8) was the first to observe and report that molybdenum and vanadium increased nitrogen fixation by azotobacter. For molybdenum the increase was as much as a hundred fold. Optimum concentration for molybdenum was found to be 1:500,000 and for vanadium 1:100,000,000 up to 1:250,000. Steinberg (70) using purified nutrient solutions also found that molybdenum stimulated nitrogen-fixation by azotobacter. Horner et al (35) found that different strains of Azotobacter vinelandii varied considerably in their molybdenum requirements. Horner et al (25) also showed that vanadium could actually replace molybdenum.

In 1949 Jordan and Anderson (43) found that boron had a stimulating effect upon the nitrogen-fixing powers of azotobacter. Silty clay loam, gravelly loam and sandy loam contained in pots were treated with borax at rates up to 160

pounds per acre. Upon plating onto nitrogen-free agar the soil from the treated pots gave increases in nitrogen values, up to 100 percent, over the untreated. Jordan and Anderson (43) also claimed that broth cultures inoculated with Az. chroococcum and treated with varying amounts of boron showed significant increases in nitrogen fixed after seven days incubation. The optimum amount was 3.5 to 7 p.p.m. boron. The same results were found when using agar plates inoculated with Az. chroococcum.

Greaves (27) inoculated a liquid medium with a soil low in sulfate and found the azotobacter growth to be scant and light of color. However, upon the addition of sulfates to the soil, before inoculation, a profuse brown chocolate growth could be seen. It appeared that sulfates were a stimulant for azotobacter growth. Greaves et al (31) applied varying amounts of $Na_2SO_4$, $K_2SO_4$, $CaSO_4$, $MgSO_4$, $MnSO_4$ and $Fe_2(SO_4)_3$ to soil contained in tumblers. They found: (1) $MgSO_4$ and $Fe_2(SO_4)_3$ failed to stimulate nitrogen-fixation at any concentration, (2) $MnSO_4$ had very little beneficial effect, (3) the rest of the sulfates did stimulate nitrogen-fixation. Greaves and Anderson (29) used eighteen different sulfur compounds, including inorganic and organic, and found: (1) azotobacter cannot oxidize a sulfur compound to a sulfate, (2) the only form in which azotobacter can utilize sulfur is in the sulfate form.

Winters (84) found with soil cultures in the laboratory that: (1) nitrogen fixation was stimulated by adding

50 - 100 p.p.m. sodium nitrate or calcium nitrate, (2) 500 p.p.m. of sodium nitrate decreased nitrogen fixation. Gainey (23) found that: (1) high concentrations of sodium nitrate tended to depress nitrogen-fixation and azotobacter population, (2) decrease varied with different soils, (3) the sensitivity is due to the nitrate ion and not the sodium or calcium, (4) when urea was added, nitrogen-fixation was depressed only after nitrification of the urea took place. Zoond, as reviewed by Thompson (72) found that concentrations over 300 p.p.m. of nitrate nitrogen depressed nitrogen-fixation.

Efficiency of nitrogen-fixation was shown by Hills (34) to decrease upon adding available combined nitrogen. Numbers of azotobacter increased but amount of nitrogen fixed did not increase proportionally.

Burk and Lineweaver (11), using the Warburg respirometer showed that calcium or strontium is essential to nitrogen fixation in pure cultures. Martin (57) showed that: (1) water soluble sodium and calcium contents of the soil were closely related to azotobacter activity, (2) the maximum limit for fixation in presence of sodium and calcium was around 3,000 p.p.m.

As reviewed by Stephenson (71) both Rosing and Burk showed that nitrogen-fixation was markedly improved by the presence of iron.

Lewis and Powers (49) have shown that iodine increases the growth rate of azotobacter.

It appears from the above review that azotobacter

are like many other organisms in that they require an assortment of elements for their nutrition.

(5) Importance of Non-symbiotic Nitrogen Fixation

The beneficial effect of symbiotic fixation of nitrogen under field conditions can be easily demonstrated. However, the value and the amount of nitrogen fixed non-symbiotically, under field conditions, are still subject to doubt.

They are subject to doubt because of three facts: (1) under natural conditions and farming practices the soil often receives very little of the type of energy material that can be utilized by the non-symbiotic nitrogen fixers, (2) since the soil contains a mixed bacterial flora, competition for this small amount of utilizable energy will exist, (3) if any gain in the soil nitrogen is made, it is often not large enough to be measured by ordinary quantitative analysis.

Waksman and Starkey (78) say that it has been stated that in some soils as much as 40 pounds per acre of nitrogen annually has been nonsymbiotically fixed. Lipman (53) states that under ordinary conditions non-symbiotic nitrogen-fixation may amount to no more than a few pounds per acre each year. However, under suitable conditions it may account for as much as fifty pounds of nitrogen per acre each year. Lipman (53) estimates that in the United States during 1930, there were 2,205,326,910 lbs. of non-symbiotically fixed nitrogen added to the 367,554,485 acres of harvested crop area.

This estimate approximately equals 6 pounds per acre.

Almost from the time that non-symbiotic nitrogen-fixing bacteria were first isolated scores of workers have tried to ascertain the beneficial effect of inoculating soils and crops with azotobacter. Allison (1) states that "Azotogen", which is a commercial preparation of Azotobacter chroococcum in a peat-soil-calcium-carbonate mixture, was used on 5 million acres of crops in the U.S.S.R. in 1942. Allison (1) also states that Soviet investigators began their inoculating trials about 1928, and since that time have made some startling claims. According to Allison (1) Sheloumova obtained average yield increases of 24 percent for mustard, corn and tobacco grown upon unlimed inoculated soils. For wheat and oats Savostin obtained average yield increases of 25 percent. Many soil bacteriologists in the Western Hemisphere have tried to duplicate the Russian successes but have failed.

In 1923 Gainey (21), at Kansas, began a 20 year experiment to gain information on the following points: what factors influence the longevity of azotobacter after being inoculated into a soil from which it was absent? (2) what effect has the introduced azotobacter on the soil's producing ability? The selected soil had a pH of 5.8 and from data collected during the experiment he concluded: (1) the addition of super-phosphate at the rate of 1,000 pounds per acre, did not influence the longevity of azotobacter, (2) the addition of superphosphate and lime, to give a pH of 6.5, enabled the azotobacter to survive throughout the 20 years,

(3) the azotobacter did not influence the crop yield, (4) the nitrogen balance of the soil was not affected.

Allison et al (2) conducted greenhouse experiments, dealing with the effect of azotobacter inoculation and found that inoculation gave no increase in yields for kali, barley, rape, rye and Swiss chard. For more evidence which contradicts the statements of the Soviet scientists the author refers those who are interested to Allison's (1) excellent review of the subject.

Part II - Decomposition of Plant Residue

(1) Decomposition of the Plant as a Whole

Waksman and Starkey (78) claim that immediately after the incorporation of plant residue in the soil, provided favorable conditions of moisture and aeration exist, it is attacked by a great variety of microorganisms. These microorganisms consist of bacteria, fungi, actinomycetes, protozoa, worms and insect larvae.

Waksman and Starkey (78) also state that the sequence in which the plant substances disappear by decomposition is as follows: (1) water-soluble substances, (2) starches, (3) protein, (4) hemicelluloses, (5) cellulose, (6) lignin, (7) fats, (8) waxes.

From the above statement it would appear that an increase in structural complexity of a plant substance often results in a slower bacterial decomposition.

As reviewed by Waksman and Starkey (78) the water-soluble substances such as sugars decompose within a few days.

Within 10 - 20 days only a portion of the cellulose, most of the lignin and a large part of the waxes and fats remains.

According to MacBeth and Scales (59) cellulose may be decomposed either under anaerobic or aerobic conditions. The anaerobic process falls into two groups: (1) without the presence of nitrate the cellulose may undergo a hydrogen, or methane fermentation, (2) with nitrates present denitrifying bacteria may decompose it with liberation of water and carbon dioxide. The aerobic process is divided according to the reaction of the medium: (1) if slightly alkaline certain aerobic bacteria will play the principal role, (2) if acid, the fungi are responsible for the cellulose destruction.

Stephenson (71) mentions that cellulose is broken down in two stages:

$$\text{Cellulose} \xrightarrow{\text{cellulase}} \text{Cellobiose} \xrightarrow{\text{cellobiase}} \text{glucose}.$$

The two enzymes, cellulase and cellobiase, are present in cellulose decomposing organisms.

Waksman and Starkey (78) state that only certain groups of Basidiomycetes and Actinomyces are able to attack lignin, and their action is very slow. Under anaerobic conditions lignin does not decompose.

(2) Factors Influencing Plant Decomposition

Waksman et al (79) found that pure cultures of facultative-thermophilic organisms were less active than a mixed culture when used to decompose compost.

Waksman et al (77) found that temperatures had the following influence upon the decomposition of composts:

(1) at 75°C; fungi were completely repressed. Actinomycetes were seldom present. The active bacteria were the hemicellulose decomposers, (2) at 65°C; fungi were still repressed. Thermophilic actinomycetes and bacteria were the predominant organisms. (3) at 50°C; the most rapid decomposition took place. A mixed population containing thermophilic bacteria, actinomycetes and fungi were very active, (4) at 28°C; a heterogeneous population composed of bacteria, actinomycetes, fungi, protozoa and nematods was present.

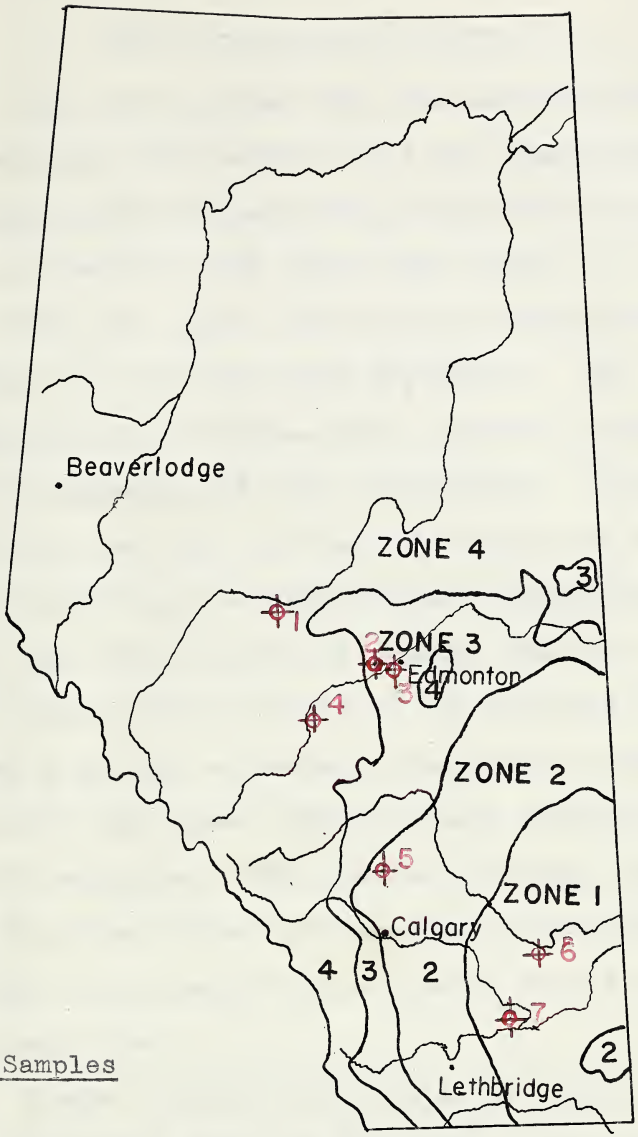
Waksman and Starkey (78) state that the optimum soil moisture for maximum bacterial development is between 50 and 70 percent of the moisture holding capacity.

Dubos (13) found that: (1) cellulose decomposing actinomycetes, in neutral soils, have an optimum moisture of 50 percent saturation, (2) cellulose decomposing bacteria in neutral soils or slightly alkaline soils have an optimum moisture of 60 to 80 percent saturation, (3) cellulose decomposing fungi have an optimum moisture of 50 to 90 percent saturation (4) in normal soils kept under water, a lag period of 14 to 16 days is required before active decomposition of cellulose takes place.

Waksman (76) reports that nitrogen has the following influence upon decomposition of plant residue: (1) cereal straws which contain 0.2 - 0.5 percent nitrogen require the addition of available nitrogen for rapid decomposition, (2) if plant residue contains more than 1.5 or 1.7 percent of nitrogen ammonia will be liberated, (3) when the nitrogen content is less than 1.5 percent very little ammonia is liberated.

Figure I

SOIL ZONES OF ALBERTA




Location of Samples

1 Blue Ridge
2 Winterburn
3 Edmonton
4 Breton
5 Acme
6 Duchess
7 Vauxhall

## PURPOSE AND OUTLINE OF INVESTIGATION

The work reported here was undertaken to determine the distribution, the activity and the beneficial action of the non-symbiotic nitrogen-fixing bacteria in some of the soils located in Alberta's four major soil zones.

The four major soil zones of Alberta are separated on the basis of soil color and vegetation. The brown soil zone occupies all of south-eastern Alberta and is characterized by low precipitation and high evaporation. To the west and north of the brown zone is the dark brown zone which is characterized by having a slightly higher precipitation, resulting in denser and taller growth of grasses than in the brown zone. The black zone, which is found to the west and north of the dark brown zone, has an average precipitation of about 18 inches and is mostly tall grass land with many scattered woodland areas within its boundaries. The grey wooded zone, lying west and north of the black zone, is characterized by a fairly cool climate and is generally fairly heavily wooded with decidious and evergreen trees.

A more detailed description of these zones may be obtained from "Soil Zones of Alberta", a map which is distributed by the Extension Department, University of Alberta.

The district and texture of the soils collected were as follows:

(1) Edmonton clay loam.

(2) Winterburn fine sandy loam.

(3) Blue Ridge fine sandy loam.

(4) Breton loam

(5) Acme loam, solonetz-like profile

(6) Duchess silt loam, solonetz-like profile

(7) Vauxhall loam

For locations and soil zones see Fig. I.

In general, the work reported in this thesis can be divided into four parts. These parts are as follows:

(1) Sand Cultures Inoculated With Soil

Sterilized sand cultures variously treated, were inoculated with soil and used to determine: (1) the relative nitrogen-fixing powers of different soils, (2) the influence of the incubation period upon nitrogen fixation, (3) the influence of soil depth upon nitrogen fixation, (5) the essentiality or the non-essentiality of taking replicate composite samples in the field to obtain a more reliable value of a soil's nitrogen-fixing power, (6) the relative quantities of nitrogen fixed when the amount of inoculum (soil) was varied, (7) the influence of mannitol concentration on nitrogen fixation.

(2) Sand Cultures Inoculated With a Soil-Straw Mixture

Here, sterilized sand cultures were inoculated with a soil-straw mixture to determine whether or not the byproducts of various decomposing straws are beneficial for nitrogen fixation.

(3) Soil Cultures Containing Straw

Various straws were added to unsterilized soil

cultures. These experiments were conducted for the purpose of gaining information on the probability of non-symbiotic nitrogen fixation under field conditions, paying particular attention to the following points: (1) does decomposing straw aid nitrogen fixation? (2) will irrigated soils fix more or less nitrogen than non-irrigated soils? (3) does water logging a soil alter nitrogen fixation?

(4) Bacteriological Examination

Attempts were made to isolate azotobacter from all soils used in these experiments. Also to obtain the relative amounts of nitrogen fixed when pure cultures of these azotobacter were inoculated into sterilized sand cultures.

# MATERIALS AND METHODS

## (1) Soil Sampling

### (a) Samples Collected For Sand Culture Inoculation and Bacteriological Examination

Where soil samples were taken from non-irrigated areas, the location was so selected that virgin and cultivated samples could be taken from adjacent soils. In the irrigated areas the virgin, the cultivated irrigated and the cultivated non-irrigated soils were sampled only if a canal separated the cultivated irrigated from the cultivated non-irrigated soil and the virgin bordered either the cultivated non-irrigated or the cultivated irrigated.

These precautions, in selecting a sampling site, were used to insure a uniformity between the virgin and cultivated soils; thus any disclosed differences in nitrogen fixation would not be the fault of soil variation, but probably due to cultural practices.

Once the site had been chosen duplicate composite samples, designated A and B, were taken at a depth of 0 - 6 inches and at a depth of 6 - 12 inches. A composite sample consisted of soil from three different holes.

All holes were dug with a shovel that had been thoroughly scoured with the soil being sampled. The exposed profile (0 - 12 inches) was then sampled, at the two above mentioned depths, with a sterilized spoon, the dislodged soil

being placed in a sterilized pan. When the pan contained the three samples (composite sample) the contents were well mixed and a suitable portion of the soil was transferred to sterilized 500 ml. erlenmyer flasks. Following the sampling the erlenmyers, with their contents, were brought to the laboratory for air-drying. The air-dried soil was then finely ground, by using a sterilized mortar and pestle, and returned to its erlenmyer.

In spite of gathering soil samples by using the above aseptic methods it was realized that because of air contamination, perfectly aseptic conditions are difficult to maintain.

(b) Samples Collected For Soil Cultures

The large samples required for this experiment made it necessary to abandon the aseptic techniques and gather the soil in jute sacks. Here grinding was accomplished in an ore pulverizer which was sterilized between samples with 5% mercuric chloride.

(2) Sand Cultures

Forty-five or fifty grams of thoroughly washed Ottawa white sand placed in 300 ml. erlenmeyer flasks were used as a base for the cultural medium. Previous to inoculating with the soil, or soil-straw culture, the flasks and contents were autoclaved at 15 pounds steam pressure for thirty minutes.

Sterilized spatulas and weighing dishes were used to add the inoculum, which was distributed amongst the sand

base by gently agitating the flask.

Following the addition of the inoculum, 10 ml. of sterile nitrogen-free solution were added to each flask. The solution medium used is that described by Fred and Waksman (19), modified to contain the following constituents:

| | |
|---|---|
| Mannite $C_6Hg(OH)_6$ | 20.0 grams |
| Magnesium sulfate $MgSO_4 \cdot 7H_2O$ | 0.2 " |
| Dipotassium phosphate $K_2HPO_4$ | 0.2 " |
| Sodium chloride NaCl | 0.2 " |
| Calcium sulfate $CaSO_4 \cdot 2H_2O$ | 0.1 " |
| Calcium carbonate $CaCO_3$ | 5.0 " |
| Distilled water | 1000.0 " |

Ten milliliters of this nutrient solution, the quantity used for the sand cultures, would contain 0.2 grams mannite. Where flasks contained 0.4 grams and 0.8 grams of mannite, the above solution contained 40.0 grams and 80.0 grams of mannite respectively. In the case where flasks contained only water plus essential inorganic elements, the above solution, without mannite, was used.

Using soil alone as the inoculum the amounts were either 0.1 grams or 0.5 grams. Six inoculated flasks, using the same amount of inoculum, were set up for each composite soil sample. Upon adding the nutrient solution two flasks were immediately sterilized to be used as controls. Generally the incubation period was 28 days. However, to ascertain what influence incubation periods had upon nitrogen fixation, a few sets were incubated 49 days and then compared with the

28 day incubated sets. All flasks were incubated in anhydro incubators where the temperature was approximately 28°C.

When a soil-straw mixture was used as the inoculum, the amount of the mixture added was 5.0 grams or 7.5 grams; here 8 flasks were inoculated and three of the eight flasks were sterilized for controls. The incubation period was always 28 days and the flasks were incubated in cupboards at room temperature.

To prepare the soil-straw inocula, two soils, Edmonton and Winterburn, were used. Five straws, namely, wheat, oats, barley, flax and alfalfa were finely ground and added at two rates. The rates were so calculated that the soil-straw mixture contained 2 and 33 percent of straw.

After the straw had been added to the soil it was necessary to thoroughly mix the two. It was found from experience (about 200 results had to be discarded) that if the original preparation was not thoroughly mixed the nitrogen contents of the controls, for each set, were very variable. However, inocula taken from well mixed soil-straw mixtures gave excellent replication.

The soil-straw inocula containing 2 percent straw were added in two conditions. The first consisted of the mixture after it had been incubated 42 days at 60 percent of the total water holding capacity. The object of this was to ascertain whether or not partly decomposed straw would have enough remaining energy sources to stimulate nitrogen fixation. The second consisted of the soil-straw inoculum immediately

after it had been mixed.

In the case of the soil-straw inoculum that contained 33 percent straw, the inoculum was not previously incubated to partly decompose the straw.

For both conditions checks (no straw added to the soil) were set up. Hence the check for the 42-day previous incubation, was soil alone that had been incubated at 60 percent of the total water holding capacity for 42 days before using as an inoculum.

After inoculating with the soil-straw mixture, four solution treatments were employed. Besides the solution treatment of water alone, water plus essential inorganic elements, and water plus essential inorganic elements plus two levels of mannite, were used. The essential inorganic elements were added to replace any element deficiency that may have been present in the soil. The mannite solutions were added to determine whether or not the nitrogen-fixing organisms could utilize energy under these conditions. Consequently mannite solutions were not generally added to all five straws. Using the soil-straw inoculum containing 2 percent straw, the mannite solutions were added only to the check (no straw), a cereal (wheat), and a legume (alfalfa). Where the soil-straw mixture inoculum contained the higher percentage of straw (33 percent) the mannite solutions were added to all straws.

All of the above mentioned inocula when placed under these apparently suitable conditions for growth should reflect the suitability of such soils or soil-straw mixture as

habitats for the nitrogen-fixing organisms.

All flasks containing sand cultures were checked every two weeks for loss of weight due to evaporation, and sterile distilled water was added to bring flasks up to their original weight.

(3) Soil Cultures Containing Straw

For these experiments finely ground straw at the rate of 5 percent was added to large quantities, generally about 3000 grams, of air-dried soil. After very thoroughly mixing the straw and soil, 130 gram portions were placed in 300 ml. erlenmyer flasks, and enough solution medium was added to bring the moisture content up to 60 percent of the water holding capacity (air-dry basis). For irrigated soils that have been accustomed to large amounts of water two levels of moisture were used; 60 percent and 105 percent of the water holding capacity (air-dry basis). The latter moisture content was used to determine the amount of nitrogen fixed under anaerobic conditions.

Immediately after adding the solution, five 5 gram portions (air-dry basis) of the soil-straw mixture were removed for nitrogen analysis. This left the flask containing 105 grams of the original air-dried soil-straw mixture. The flasks were then incubated at room temperature for 35 days at the end of which time another five replicates were removed for nitrogen analysis.

In the case of the controls (soil without straw) 125 grams were added to the flasks. Therefore, after the

removal of the quantity for nitrogen determination, the flask would contain 100 grams of soil (air-dry basis).

Since these large samples of soil should contain numerous nitrogen-fixing organisms, the solution medium was so calculated that each 105 gm. of the soil-straw mixture and each 100 gm. of soil alone (check) received twice as much of the essential inorganic elements as the sand cultures. Where mannite was added the rates were 0.8 gm. and 1.6 gm. per flask.

For every flask set up in these experiments an identical amount of incubating material (105 grams for the straw treated and 100 grams for the control) were placed in "Gem" quart sealers. The purpose of this latter set was to determine the amount of carbon dioxide evolved during the incubation period.

(4) Bacteriological Examination

For the isolation of azotobacter, about 2 grams of soil were inoculated into 20 ml. of the above solution contained in 300 ml.erlenmyer flasks. After about three days the culture which developed in the medium was sub-cultured into fresh medium and the process repeated two or three times. At this stage the culture was plated onto nitrogen-free mannite agar plates. Any typical azotobacter colonies then found were transferred to slants for refrigerator storage.

The controls consisted of the same additives, only here the flask was sterilized after the soil inoculation and then seeded with 1 ml. of a pure azotobacter culture. In this

way it was possible to ascertain from the absence or presence of growth whether the non-presence, which frequently occured, of azotobacter was due to adverse conditions present in the solution.

(5) Analytical Methods and Calculations

(a) Nitrogen Determinations

Total nitrogen (organic nitrogen plus nitrate nitrogen) was determined by the modified Kjeldahl method, Lincoln and Walton (50), making use of zinc dust as the nitrate reducer. The boric acid method of Scales and Harrison (67) was used for the recovery of the distilled ammonia. For the sand cultures containing 0.1 and 0.5 grams of inoculum N/50 sulfuric acid was used to make the titrations. In the case of 5 gram soil-straw inocula N/14 sulfuric acid was used.

The average for the controls, in each set, were subtracted from each treated flask to obtain the amount of nitrogen fixed in each flask.

(b) Determination of Carbon Dioxide Evolved and Organic Matter Lost in Soil Cultures

The method of determining the amount of carbon dioxide evolved by soil cultures during their 35 day incubation period was essentially that used by Norman and Lewis (61). The evolved carbon dioxide was absorbed by N/2 NaOH contained in 4-oz. bottles resting on short glass rods which were on the soil surface. To make quart sealers air tight the tops were screwed on tightly.

Each day for the first two weeks, followed by every

other day for the remainder of the incubation period, the 4-oz. bottles were removed and $BaCl_2$ was added to precipitate the carbonates. The excess base was then titrated with N/2 HCl. The sealers were left open while fresh NaOH was being added to the 4-oz bottles thus allowing fresh air to enter and insure an adequate supply of oxygen.

The amount of carbon dioxide given off by the soil in a 24 or 48-hour period was calculated; one cubic centimeter of N/2 NaOH will absorb 0.0110 grams of carbon dioxide.

Many authors including Lunt (56) state that multiplying carbon dioxide by the conventional factor 0.471 is the most reliable method for determining the amount of organic matter present in soils. Its reliability is based upon the fact that soil organic matter averages close to 58 percent in content of carbon. Consequently this method was used to determine the amount of organic matter lost by the soil cultures during their incubation period.

The apparent necessity of knowing the amount of organic matter lost can be clarified by the following hypothetical example: Suppose 100 grams of a soil-straw mixture contains 0.400 grams of nitrogen. Here the percentage nitrogen would be 0.400 percent. After incubation the soil-straw mixture may have lost 3.0 grams of organic matter and still not have gained in nitrogen content; thus upon analysis one would find $\frac{0.400}{97} \times 100 = 0.412$ percent nitrogen.

This would represent an erroneous increase in nitrogen and probably would be attributed to nitrogen fixation.

To make the necessary corrections the author determined: (1) the oven dry wt. of the air dry soil-straw mixture being placed in the flasks, (2) the amount of organic matter lost by this soil-straw mixture using the above method.

An example of the calculations is as follows:

(1) 105.0 grams of air dry soil-straw mixture weighs 102.4 grams (oven dry).

(2) During the 35 day incubation period the soil lost 5.82 grams of $CO_2$ or 5.82 x 0.471 = 2.74 grams of organic matter.

(3) After incubation this soil-straw mixture would have weighed 102.4 - 2.74 = 99.66 grams (oven dry).

(4) Therefore, each oven dried gram, after incubation, analyzed for nitrogen should have weighed $1 \times \frac{102.4}{99.6} = 1.028$ grams before incubation.

(5) A five gram sample (the approximate amount used for analysis) would have weighed 5 x 1.028 = 5.140 grams before incubation.

(c) Statistical Analysis

Statistical analyses for data presented in this thesis were computed as described by Goulden (26).

## RESULTS AND DISCUSSION

Except for Table I the results presented and discussed here are divided into the same sections as mentioned under the heading, "Purpose and Outline of Investigation."

Table I presents the results of two laboratory analyses which were conducted on the soils under study. Although it is far from being a complete analysis, it gives the two factors which largely affect nitrogen fixation: (1) the reaction of the soil, and (2) the nitrogen content of the soil. The reaction, as surveyed in the literature review, is useful in corroborating the absence or presence of azotobacter. The nitrogen content of a soil should affect the quantity of nitrogen fixed for as the quantity of available nitrogen increases the amount fixed decreases.

Virgin and cultivated soils (see Table I) from the brown zone have an average pH of 7.3 and 7.2 respectively. For the dark brown zone the virgin and cultivated soil averaged 7.0 and 7.1 respectively. For the black zone the average pH is 6.3 and 6.0 respectively, and for the grey-wooded soil zone the average pH is 5.5 and 5.1 respectively. These average pH results would indicate that only the soils from the brown and dark brown zones fall well within the range of pH 6.5 - 8.6 given by Fred and Davenport (18) as optimum for aerobic nitrogen-fixing bacteria.

For all soils the nitrogen content averages 63 percent higher for the virgin than for the cultivated soil. This great

TABLE I. Chemical Analyses of Original Soil Samples

Used for Nitrogen Fixation Studies (average of duplicate determinations)

| Zone | Soil | Virgin or Cult. | Soil Depth | pH | Nitrogen % |
|---|---|---|---|---|---|
| Brown | Vauxhall loam | Virgin | 0" - 6" | 7.3 | 0.161 |
| " | " " | " | 6" - 12" | 7.3 | 0.120 |
| " | " " | Cult. Irr. | 0" - 6" | 6.9 | 0.179 |
| " | " " | " " | 6" - 12" | 7.5 | 0.112 |
| " | " " | Cult. Non-Irr. | 0" - 6" | 7.5 | 0.152 |
| " | " " | " " " | 6" - 12" | 7.6 | 0.103 |
| " | Duchess silt loam | Virgin | 0" - 6" | 6.6 | 0.168 |
| " | " " " | " | 6" - 12" | 7.9 | 0.087 |
| " | " " " | Cult. Irr. | 0" - 6" | 6.8 | 0.208 |
| " | " " " | " " | 6" - 12" | 7.5 | 0.093 |
| " | " " " | Cult. Non-Irr. | 0" - 6" | 7.0 | 0.211 |
| " | " " " | " " " | 6" - 12" | 6.8 | 0.151 |
| Dark Brown | Acme loam | Virgin | 0" - 6" | 5.7 | 0.408 |
| " " | " " | " | 6" - 12" | 7.2 | 0.234 |
| " " | " " | Cult. | 0" - 6" | 6.9 | 0.250 |
| " " | " " | " | 6" - 12" | 7.3 | 0.105 |
| Black | Winterburn fine sandy loam | Virgin | 0" - 6" | 6.0 | 0.259 |
| " | " " " " | " | 6" - 12" | 6.2 | 0.164 |
| " | " " " " | Cult. | 0" - 6" | 6.1 | 0.241 |
| " | " " " " | " | 6" - 12" | 6.0 | 0.105 |
| " | Edmonton clay loam | Virgin | 0" - 6" | 5.9 | 0.994 |
| " | " " " | " | 6" - 12" | 6.9 | 0.485 |
| " | " " " | Cult. | 0" - 6" | 5.9 | 0.552 |
| " | " " " | " | 6" - 12" | 5.9 | 0.222 |
| Grey Wooded | Blue Ridge fine sandy loam | Virgin | 0" - 6" | 5.8 | 0.093 |
| " " | " " " " " | " | 6" - 12" | 5.4 | 0.053 |
| " " | " " " " " | Cult. | 0" - 6" | 5.4 | 0.134 |
| " " | " " " " " | " | 6" - 12" | 5.5 | 0.041 |
| " " | Breton loam | Virgin | 0" - 6" | 5.3 | 0.205 |
| " " | " " | " | 6" - 12" | 5.4 | 0.042 |
| " " | " " | Cult. Check | 0" - 6" | 5.4 | 0.099 |
| " " | " " | " " | 6" - 12" | 5.2 | 0.071 |
| " " | " " | Cult. $(NH_4)_2SO_4$ | 0" - 6" | 4.9 | 0.122 |
| " " | " " | " " | 6" - 12" | 4.9 | 0.090 |
| " " | " " | Cult. complete | 0" - 6" | 4.8 | 0.118 |
| " " | " " | " " | 6" - 12" | 4.9 | 0.074 |

difference, especially where five grams is used as the amount of inoculum, may account for less nitrogen being fixed in the virgin than in the cultivated.

(1) Sand Cultures Inoculated With Soil

The results of these experiments are presented in Tables II -VIII. Quadruplicate results are given in order to reveal the degree of variation evident between cultures. Each figure represents the amount of nitrogen fixed per flask after deducting the amount present in the sterilized controls.

In Table II are the results of adding 0.5 gm. of Edmonton, Winterburn and Breton soils to sand cultures, receiving 10 ml. of a solution containing essential inorganic elements and 0.2 gm. of mannite. The analysis of variance carried out on the milligrams of nitrogen fixed per gram of mannite indicate that duplicate differences are not significant. This would imply that single composite samples are as reliable as duplicate composite samples when determining the nitrogen fixing power of a soil. Or in other words the nitrogen-fixing organisms of a given soil probably do not vary significantly in numbers and kinds over a small area. The differences in the amount of nitrogen fixed by the three soils were not statistically significant. The statistical analyses reveal that cultivated soil from these three areas are significantly better nitrogen-fixing habitats than the virgin soils. The explanation for this difference may be: (1) since conditions in the sand cultures are largely aerobic, nitrogen-fixing organisms from cultivated areas have become better adapted to these conditions,

TABLE II

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubation for Periods of 28 Days and 49 Days at 28°C.

One-half gm. portions of Edmonton, Winterburn and Breton soils were mixed with 50 gm. sand and treated with 10 ml. of a solution containing essential inorganic elements and 0.2 gm. mannite.

| Soil | Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" 28 days | 0" - 6" 49 days | 6" - 12" 28 days | 6" - 12" 49 days | Ave. Type Cult. | Ave. Type Virgin |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 7.44 | 5.49 |
| Edm. | Cultivated | A | 7.95 | 4.56 | 4.91 | 6.83 | | |
| | | | 6.94 | 7.24 | 5.92 | 9.11 | | |
| | | | 7.70 | 6.38 | 8.45 | 8.10 | | |
| | | | 5.16 | 7.24 | 9.47 | 7.85 | | |
| | | Ave. | 6.94 | 6.36 | 7.19 | 7.97 | | |
| | | B | 6.43 | 6.33 | 9.97 | 7.19 | | |
| | | | 6.68 | 6.08 | 8.96 | 9.47 | | |
| | | | 7.19 | 6.83 | 9.47 | 6.94 | | |
| | | | 7.70 | 6.95 | 8.20 | 8.20 | | |
| | | Ave. | 7.00 | 6.55 | 9.15 | 7.97 | | |
| | | Ave. Soils x Types x Depths | 6.71 | | 8.07 | | | |
| | Virgin | A | 5.67 | 5.82 | 5.32 | 5.06 | | |
| | | | 4.90 | 5.57 | 4.56 | 5.06 | | |
| | | | 4.91 | 5.57 | 5.57 | 5.06 | | |
| | | | 4.00 | 5.60 | 5.32 | 5.57 | | |
| | | Ave. | 5.87 | 5.64 | 5.15 | 5.15 | | |
| | | B | 4.27 | 4.30 | 3.79 | 5.56 | | |
| | | | 3.29 | 4.30 | 4.55 | 5.81 | | |
| | | | 4.02 | 5.06 | 0.55 | 4.30 | | |
| | | | 5.74 | 4.56 | 4.55 | 5.39 | | |
| | | Ave. | 4.33 | 4.56 | 2.86 | 5.27 | | |
| | | Ave. Soils x Types x Depths | 4.85 | | 4.75 | | | |
| Wint. | Cultivated | A | 8.45 | 9.37 | 7.70 | 8.10 | | |
| | | | 6.18 | 7.89 | 8.20 | 7.09 | | |
| | | | 8.70 | 8.65 | 8.20 | 7.59 | | |
| | | | 6.18 | 8.65 | 6.68 | 7.09 | | |
| | | Ave. | 6.85 | 8.69 | 7.70 | 7.47 | | |
| | | B | 6.43 | 8.10 | 6.33 | 5.57 | | |
| | | | 6.18 | 7.34 | 7.09 | 6.58 | | |
| | | | 7.00 | 8.66 | 5.57 | 6.33 | | |
| | | | 7.09 | 7.74 | 4.30 | 6.58 | | |
| | | Ave. | 6.70 | 7.96 | 5.82 | 6.27 | | |
| | | Ave. Soils x Types x Depths | 7.66 | | 6.81 | | | |
| | Virgin | A | 7.19 | 8.65 | 6.99 | 5.47 | | |
| | | | 6.68 | 8.35 | 6.68 | 6.68 | | |
| | | | 6.18 | 7.59 | 6.99 | 8.45 | | |
| | | | 5.47 | 6.58 | 6.99 | 5.16 | | |
| | | Ave. | 6.38 | 7.79 | 6.91 | 6.44 | | |
| | | B | 7.89 | 7.59 | 8.35 | 8.86 | | |
| | | | 7.59 | 7.59 | 6.33 | 7.34 | | |
| | | | 7.09 | 6.08 | 6.32 | 8.35 | | |
| | | | 7.59 | 6.58 | 6.32 | 6.83 | | |
| | | Ave. | 7.54 | 6.96 | 6.81 | 7.85 | | |
| | | Ave. Soils x Types x Depths | 7.17 | | 7.01 | | | |
| Breton | Cultivated | A | 8.10 | 6.18 | 7.34 | 7.95 | | |
| | | | 6.33 | 5.42 | 9.87 | 5.92 | | |
| | | | 9.87 | 7.29 | 9.11 | 7.44 | | |
| | | | 9.67 | 6.78 | 7.59 | 4.91 | | |
| | | Ave. | 8.49 | 6.42 | 8.48 | 6.56 | | |
| | | B | 9.47 | 8.96 | 9.11 | 7.44 | | |
| | | | 8.00 | 8.45 | 7.34 | 8.45 | | |
| | | | 7.70 | 6.68 | 5.57 | 8.70 | | |
| | | | 8.52 | 6.68 | 7.34 | 7.95 | | |
| | | Ave. | 8.42 | 7.69 | 7.33 | 8.15 | | |
| | | Ave. Soils x Types x Depths | 7.76 | | 7.63 | | | |
| | Virgin | A | 4.66 | 5.32 | 3.29 | 4.30 | | |
| | | | 6.43 | 5.82 | 6.33 | 0.38 | | |
| | | | 4.91 | 6.83 | 4.66 | 5.06 | | |
| | | | 5.33 | 5.57 | 4.56 | 3.20 | | |
| | | Ave. | 5.33 | 5.89 | 4.71 | 3.24 | | |
| | | B | 5.06 | 4.81 | 2.28 | 4.56 | | |
| | | | 3.80 | 5.32 | 1.77 | 2.53 | | |
| | | | 4.81 | 5.06 | 2.89 | 5.82 | | |
| | | | 5.32 | 4.06 | 5.90 | 5.57 | | |
| | | Ave. | 4.75 | 4.81 | 3.21 | 4.62 | | |
| | | Ave. Soils x Types x Depths | 5.19 | | 3.94 | | | |

## Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 1.41 | |
| Soils | 2 | 23.56 | |
| Types (Field Treatment) | 1 | 183.16 x | 1.43 |
| Depths | 1 | 1.72 | |
| Incubations (28 and 49 days) | 1 | 0.40 | |
| Soils x Types | 2 | 40.16 | |
| Soils x Depths | 2 | 8.16 | |
| Soils x Incubations | 2 | 3.63 | |
| Types x Depths | 1 | 4.73 | |
| Types x Incubations | 1 | 4.41 | |
| Depths x Incubations | 1 | 0.00 | |
| Soils x Types x Depths | 2 | 5.32 x | 0.78 |
| Soils x Types x Incubations | 2 | 2.91 | |
| Soils x Depths x Incubations | 2 | 1.27 | |
| Types x Depths x Incubations | 1 | 0.21 | |
| Soils x Types x Depths x Incubations | 2 | 1.30 | |
| Error | 175 | 1.24 | |
| Total | 191 | | |

(2) cultivation may have introduced more or better strains of nitrogen-fixing organisms, or (3) the lower nitrogen content of cultivated soil induces the nitrogen-fixing organisms to utilize atmospheric nitrogen. There was markedly less nitrogen fixed in the virgin than the cultivated soils for Edmonton and Breton, but there was less difference in the case of Winterburn. This differential response was not statistically significant. Although depths are not significantly different, the data do suggest that the depths influence nitrogen fixation in sand cultures. This is emphasized by the fact that the 0" - 6" depth for Winterburn cultivated and Breton virgin is significantly better than the 6" - 12" depth. For Edmonton cultivated the reverse is true. This change in trend is reflected in soils x types x depths being statistically significant. The reverse condition occuring in the Edmonton cultivated may indicate that the lower depth for Edmonton cultivated is just as fertile as, or more fertile than, the upper depth. In the case where incubation is significant it suggests that a 28-day incubation period is sufficient to determine the amount of nitrogen fixed by these organisms.

Table III presents the data where 0.5 gm. of Breton soil was used as inoculum. This experiment was designed to determine whether or not fertilization enhances nitrogen fixation. Duplicates again are not statistically different. Since types are not statistically different it is apparent, using this method of determination, that fertilization does not stimulate nitrogen fixation. Unlike the data of Table II where

TABLE III

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubation for Periods of 28 Days and 49 Days at 28°C.

One-half gm. portions of Breton soil were mixed with 50 gm. sand and treated with 10 ml. of a solution containing essential inorganic elements and 0.2 gm. mannite. The soil was obtained from the cultivated Series A (grain-legume rotation) and the adjacent virgin headland at the Breton Experimental Field. The three sampled cultivated plots numbers 3, 4 and 5 had received complete fertilizer, $(NH_4)_2SO_4$ and no fertilizer respectively, every two years, since 1930.

| Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" 28 days | 0" - 6" 49 days | 6" - 12" 28 days | 6" - 12" 49 days | Ave. Types x Incubations 28 days | Ave. Types x Incubations 49 days |
|---|---|---|---|---|---|---|---|
| Cultivated No Fertilizer | A | 8.10 | 6.18 | 7.34 | 7.95 | | |
| | | 6.33 | 5.42 | 9.87 | 5.92 | | |
| | | 9.87 | 7.29 | 9.11 | 7.44 | | |
| | | 9.67 | 6.78 | 7.59 | 4.91 | | |
| | Ave. | 8.49 | 6.42 | 8.48 | 6.56 | | |
| | B | 9.47 | 8.96 | 9.11 | 7.44 | | |
| | | 8.00 | 8.45 | 7.34 | 8.45 | | |
| | | 7.70 | 6.68 | 7.34 | 7.95 | | |
| | | 8.52 | 6.68 | 7.34 | 7.95 | | |
| | Ave. | 8.42 | 7.69 | 7.33 | 8.15 | 8.18 | 7.20 |
| Cultivated $(NH_4)_2SO_4$ | A | 8.35 | 6.58 | 6.08 | 6.83 | | |
| | | 7.59 | 10.38 | 5.06 | 6.58 | | |
| | | 7.34 | 7.85 | 5.57 | 6.58 | | |
| | | 7.76 | 9.13 | 6.83 | 6.83 | | |
| | Ave. | 7.76 | 8.49 | 5.89 | 6.71 | | |
| | B | 5.36 | 6.68 | 4.05 | 5.06 | | |
| | | 6.58 | 8.71 | 7.09 | 8.10 | | |
| | | 5.06 | 7.70 | 5.06 | 5.06 | | |
| | | 5.82 | 7.20 | 5.06 | 9.62 | | |
| | Ave. | 5.71 | 7.82 | 5.32 | 6.92 | 6.17 | 7.49 |
| Cultivated Complete Fertilizer | A | 7.59 | 6.43 | 6.58 | 5.82 | | |
| | | 8.10 | 7.19 | 6.33 | 6.33 | | |
| | | 6.58 | 6.18 | 6.33 | 7.09 | | |
| | | 9.11 | 6.60 | 7.34 | 7.59 | | |
| | Ave. | 7.85 | 6.60 | 6.65 | 6.71 | | |
| | B | 7.90 | 8.35 | 5.41 | 4.30 | | |
| | | 7.09 | 8.35 | 9.97 | 6.58 | | |
| | | 6.08 | 8.35 | 5.16 | 6.58 | | |
| | | 6.58 | 7.59 | 6.18 | 7.07 | | |
| | Ave. | 6.91 | 8.16 | 6.68 | 6.13 | 7.02 | 6.90 |
| Virgin | A | 4.66 | 5.32 | 3.29 | 4.30 | | |
| | | 6.43 | 5.82 | 6.33 | 0.38 | | |
| | | 4.91 | 6.83 | 4.66 | 5.06 | | |
| | | 5.33 | 5.57 | 4.56 | 3.20 | | |
| | Ave. | 5.33 | 5.89 | 4.71 | 3.25 | | |
| | B | 5.06 | 4.81 | 2.28 | 4.56 | | |
| | | 3.80 | 5.32 | 1.77 | 2.53 | | |
| | | 4.81 | 5.06 | 2.89 | 5.82 | | |
| | | 5.32 | 4.06 | 5.90 | 5.57 | | |
| | Ave. | 4.75 | 4.81 | 3.21 | 4.62 | 4.50 | 4.60 |
| | Ave. Depth | 6.94 | | 6.08 | | | |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 1.18 | |
| Types (Field Treatment) | 3 | 58.33** | |
| Depths | 1 | 23.74** | 0.44 |
| Incubations (28 and 49 days) | 1 | 0.26 | |
| Types x Depths | 3 | 2.19 | |
| Types x Incubations | 3 | 7.28** | 0.89 |
| Depths x Incubations | 1 | 0.00 | |
| Types x Depths x Incubations | 3 | 0.61 | |
| Error | 111 | 1.60 | |
| Total | 127 | | |

three soils were used, it may be seen that where the analysis is based only on Breton soil there is a statistically significant difference for depths. Apparently the 0" - 6" depth is superior to the 6" - 12" depth. Reasons for this difference may be: (1) as compared to Edmonton and Winterburn, Breton soil is a relatively unfertile soil. Consequently the trace elements that are needed for nitrogen-fixation, and not supplied by the nutrient solution, are more concentrated in the top six inches, (2) Breton soil is located in a relatively cool humid climate, hence the nitrogen-fixing organisms in the lower depths are not as efficient at fixing nitrogen at 28°C. as the organisms in the upper depth. This adaption to temperature has been pointed out by Greene (33) using Arizona soils, (3) Breton loam is the type of soil that bakes and puddles easily, therefore the nitrogen-fixing organisms from the upper depths have become better adapted to grow under aerobic conditions. Again incubations (28 and 49 days) are not statistically different. During the 49-day incubation period the ammonium sulphate treated plot and the virgin soil fixed more nitrogen than during the 28 day period. For the unfertilized and complete fertilizer treated plots the reverse occured. This is reflected in types x incubations being highly significant, but no explanation is apparent.

Table IV presents the data using 0.1 gm. of Breton soil as inoculum. These results were not compared statistically with the results of Table III (0.5 gm. of inoculum). However, a comparison of both tables will reveal that the 0.5 gm. amount

TABLE IV

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubations for Periods of 28 Days at 28°C.

One-tenth gm. portions of Breton Soil were mixed with 50 gm. sand and treated with 10 ml. of a solution containing essential inorganic elements and 0.2 gm. mannite. The soil was obtained from the cultivated Series A (grain-legume rotation) and the adjacent virgin headland at the Breton Experimental Field. The three sampled cultivated plots numbers 3, 4 and 5 had received complete fertilizer, $(NH_4)_2SO_4$ and no fertilizer respectively, every two years, since 1930.

| Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" | Field Sample Depth 6" - 12" |
|---|---|---|---|
| Cultivated, No Fertilizer | A | -0.40 | -0.10 |
| | | -0.15 | 0.80 |
| | | 0.55 | 0.00 |
| | | -0.30 | -0.20 |
| | Ave. | -0.08 | 0.13 |
| | B | 0.70 | 0.30 |
| | | -0.35 | 0.40 |
| | | 0.00 | -0.10 |
| | | 0.10 | -0.45 |
| | Ave. | 0.11 | 0.15 |
| | Ave. Depths x Types | 0.02 | 0.09 |
| Cultivated, $(NH_4)_2SO_4$ | A | -0.10 | -0.10 |
| | | -0.20 | -0.20 |
| | | -0.10 | 0.40 |
| | | -0.10 | 0.05 |
| | Ave. | -0.13 | 0.04 |
| | B | -1.15 | -0.60 |
| | | -0.25 | 0.60 |
| | | -1.25 | -0.35 |
| | | -1.15 | -0.10 |
| | Ave. | -0.95 | -0.11 |
| | Ave. Depths x Types | -0.54 | -0.04 |
| Cultivated, Complete Fertilizer | A | 0.25 | -0.65 |
| | | 0.60 | 0.20 |
| | | 0.80 | -0.55 |
| | | 0.80 | 0.75 |
| | Ave. | 0.61 | -0.06 |
| | B | -0.05 | -0.55 |
| | | 0.90 | -0.20 |
| | | 0.40 | -0.35 |
| | | 1.10 | -0.45 |
| | Ave. | 0.59 | -0.39 |
| | Ave. Depths x Types | 0.60 | -0.23 |
| Virgin | A | -0.35 | 0.00 |
| | | 0.10 | -0.20 |
| | | 1.15 | -0.20 |
| | | 0.35 | -0.10 |
| | Ave. | 0.31 | -0.13 |
| | B | 0.05 | 0.05 |
| | | -0.15 | -0.30 |
| | | -0.15 | 0.10 |
| | | 0.40 | 0.30 |
| | Ave. | 0.15 | 0.15 |
| | Ave. Depths x Types | 0.18 | -0.05 |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 0.45 | |
| Types (Field Treatment) | 3 | 0.66 | |
| Depths | 1 | 0.23 | |
| Duplicates x Types | 3 | 0.22 | |
| Duplicates x Depths | 1 | 0.07 | |
| Types x Depths | 3 | 1.23xx | 0.40 |
| Duplicates x Types x Depths | 3 | 0.25 | |
| Error | 48 | 0.16 | |
| Total | 63 | | |

of inoculum has given a 20-fold increase in nitrogen fixation. The reason for the difference may be : (1) the smaller amount of inoculum (0.1 gm.) would have resulted in fewer nitrogen-fixing organisms being introduced; thus competitive organisms have become predominant. However considering the fact that the solution medium is of an elective nature this explanation may be questioned, (2) using an unfertile soil such as Breton, 0.1 gm. of soil does not supply a sufficient amount of the necessary trace elements for maximum nitrogen fixation. Further evidence that a lack of trace elements caused the difference is emphasized by depths differences not being statistically significant for Table IV (0.1 gm. of inoculum), whereas in Table III (0.5 gm. of inoculum) depths differences were statistically significant. The statistical data of Table IV also reveals that fertilization is not beneficial for nitrogen fixation. Types have responded differently to depths, however, but no explanation can be offered.

Table V presents the data using 0.1 gm. of Winterburn and Edmonton soil as inoculum. Although the difference is not as marked as in Breton soil, there is definitely a decrease in nitrogen fixed where 0.1 gm. inoculum is used instead of 0.5 gm. (Table II). For Edmonton soil the average decrease is fairly large while for Winterburn it is a great deal larger. The difference in the soils, where 0.1 gm. is used, is also revealed in the fact that soil differences are highly statistically significant for the data in Table V. Because of its higher fertility it would be expected that Edmonton soil should

TABLE V

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubation for a Period of 28 Days at 28°C.

One-tenth gm. portions of Winterburn and Edmonton soils were mixed with 50 gm. sand and treated with 10 ml. of a solution containing essential inorganic elements and 0.2 gm. mannite.

| Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" Wint. | 0" - 6" Edm. | 6" - 12" Wint. | 6" - 12" Edm. | Ave. Soils Wint. | Ave. Soils Edm. |
|---|---|---|---|---|---|---|---|
| Cultivated | A | 3.50 | 8.80 | 2.20 | 1.00 | 1.37 | 3.98 |
| | | 6.15 | 6.50 | 0.80 | 3.30 | | |
| | | 5.60 | 5.20 | 0.55 | 0.20 | | |
| | | 0.20 | 4.65 | 2.80 | 4.10 | | |
| | Ave. | 3.86 | 6.29 | 1.59 | 2.15 | | |
| | B | 3.85 | 9.30 | 0.33 | 7.55 | | |
| | | 0.90 | 8.45 | 0.09 | 2.00 | | |
| | | 7.15 | 6.75 | 0.07 | 8.05 | | |
| | | 3.95 | 6.40 | 0.11 | 0.70 | | |
| | Ave. | 3.96 | 7.73 | 0.15 | 4.58 | | |
| | Ave. Types x Depths | 5.46 | | 2.16 | | | |
| Virgin | A | 0.09 | 0.35 | 0.15 | 4.65 | | |
| | | 0.26 | 0.45 | 0.08 | 3.70 | | |
| | | 0.07 | 0.60 | 0.02 | 3.45 | | |
| | | 0.74 | 3.00 | 0.15 | 3.50 | | |
| | Ave. | 0.29 | 1.10 | 0.10 | 3.83 | | |
| | B | 0.63 | 1.95 | 0.26 | 4.70 | | |
| | | 0.56 | 3.15 | 0.42 | 2.25 | | |
| | | 0.70 | 2.45 | 0.42 | 4.70 | | |
| | | 0.35 | 1.75 | 0.56 | 3.80 | | |
| | Ave. | 0.56 | 2.33 | 0.42 | 3.86 | | |
| | Ave. Types x Depths | 1.07 | | 2.05 | | | |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 4.78 | |
| Types (Field Treatment) | 1 | 79.41 | |
| Depths | 1 | 22.31 | |
| Soils | 1 | 109.44xx | 0.81 |
| Types x Depths | 1 | 74.84xx | 1.14 |
| Types x Soils | 1 | 0.51 | |
| Depths x Soils | 1 | 2.89 | |
| Types x Depths x Soils | 1 | 8.40 | |
| Error | 55 | 2.61 | |
| Total | 63 | | |

have the least decrease. Also of interest is the fact that the 0" - 6" depth for Winterburn and Edmonton cultivated soil is significantly better than the 6" - 12" depth, while in the virgin, although not significant, the reverse is true. This is pointed out by types x depths being statistically significant. The stimulatory effect of cultivation is the probable explanation for the upper depth in the cultivated soil being better than the lower depth. The higher nitrogen content of the virgin soil's upper depth may account for the reverse finding in these soils.

Table VI presents the data using 0.1 gm. of Edmonton soil as inoculum. The flasks received solutions containing two levels of mannite; 0.2 gm. and 0.4 gm. The experiment was designed to ascertain how efficiently nitrogen-fixing organisms from Edmonton soil utilize mannite.

The statistical analysis reveals that the nitrogen-fixing organisms from Edmonton soil in a mixed bacterial flora use mannite more efficiently when the smaller concentration is used. This difference is highly significant. It should be observed that the same average amount of nitrogen (see ave. solution) was fixed per flask, 0.816 milligrams, for both 0.2 gm. and 0.4 gm. of mannite. This would indicate that 0.2 gm. of mannite supplies as much energy for nitrogen fixation as 0.4 gm. of mannite. As in Table V the trend for the differential response of types x depths is the same.

Table VII presents the data for comparing the amount of nitrogen fixed by Blue Ridge and Acme soils. Although not

TABLE VI

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubation for a Period of 28 Days at 28°C.

One-tenth gm. portions of Edmonton soils were mixed with 50 gm. sand and treated with 10 ml. of solutions containing essential inorganic elements and two levels of mannite. The two levels of mannite were 0.2 gm. and 0.4 gm.

| Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" 0.2 gm. Man. | 0" - 6" 0.4 gm. Man. | 6" - 12" 0.2 gm. Man. | 6" - 12" 0.4 gm. Man. | Ave. Solution 0.2 gm. Man. | Ave. Solution 0.4 gm. Man. |
|---|---|---|---|---|---|---|---|
| Cultivated | A | 5.80 | 3.83 | 1.00 | 1.85 | 4.08 | 2.04 |
| | | 6.50 | 5.33 | 3.30 | 0.78 | | |
| | | 5.20 | 3.65 | 0.20 | 0.23 | | |
| | | 4.65 | 1.90 | 4.10 | 1.55 | | |
| | Ave. | 6.29 | 3.90 | 2.15 | 1.10 | | |
| | B | 9.30 | 2.59 | 7.55 | 2.85 | | |
| | | 8.45 | 5.49 | 2.00 | 1.51 | | |
| | | 6.75 | 4.93 | 8.05 | 1.73 | | |
| | | 6.40 | 3.95 | 0.70 | 1.10 | | |
| | Ave. | 7.73 | 4.24 | 4.58 | 1.80 | | |
| | Ave. Types x Depths | 5.48 | | 2.41 | | | |
| Virgin | A | 0.35 | 2.10 | 4.65 | 2.10 | | |
| | | 3.45 | 1.58 | 3.70 | 2.95 | | |
| | | 0.60 | 1.58 | 3.45 | 1.45 | | |
| | | 3.00 | 1.68 | 3.50 | 1.75 | | |
| | Ave. | 1.85 | 1.75 | 3.83 | 2.06 | | |
| | B | 1.95 | 0.70 | 4.70 | 0.13 | | |
| | | 3.15 | 0.53 | 2.25 | 0.23 | | |
| | | 2.45 | 0.10 | 4.70 | 3.78 | | |
| | | 1.75 | 0.12 | 3.80 | 1.05 | | |
| | Ave. | 2.33 | 0.36 | 3.86 | 1.29 | | |
| | Ave. Types x Depths | 1.57 | | 2.76 | | | |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 3.05 | |
| Types (Field Treatment) | 1 | 50.66 | |
| Depths | 1 | 14.18 | |
| Solutions (0.2 and 0.4 Man. gm.) | 1 | 66.63** | 0.76 |
| Types x Depths | 1 | 72.93** | 1.08 |
| Types x Solutions | 1 | 3.09 | |
| Depths x Solutions | 1 | 0.00 | |
| Types x Depths x Solutions | 1 | 5.11 | |
| Error | 55 | 2.31 | |
| Total | 63 | | |

TABLE VII

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubation for a Period of 28 Days at 28°C

One-tenth gm. portions of Blue Ridge (B.R.) and Acme soils were mixed with 50 gm. sand and treated with 10 ml. of a solution containing essential inorganic elements and 0.2 gm. mannite.

| Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" | | Field Sample Depth 6" - 12" | |
|---|---|---|---|---|---|
| | | B. R. | Acme | B. R. | Acme |
| Cultivated | A | 0.40 | 2.65 | 0.25 | -0.35 |
| | | 0.60 | 2.95 | 0.00 | 0.55 |
| | | -0.55 | 0.05 | 0.25 | 0.45 |
| | | 0.10 | 0.40 | -0.70 | -0.20 |
| | Ave. | 0.14 | 1.51 | -0.05 | 0.11 |
| | B | -0.20 | 2.60 | 0.20 | 0.00 |
| | | 0.15 | 4.45 | -0.15 | 0.10 |
| | | 0.15 | 0.95 | -0.15 | -0.10 |
| | | 0.75 | 3.30 | 0.40 | -0.05 |
| | Ave. | 0.21 | 2.83 | 0.08 | -0.01 |
| | Ave. Types x Depths x Soils | 0.18 | 2.17 | 0.01 | 0.05 |
| Virgin | A | 0.25 | 0.35 | 0.40 | 0.90 |
| | | 0.60 | 1.20 | 0.55 | 0.10 |
| | | 0.25 | 0.65 | 0.10 | 0.70 |
| | | 0.55 | 0.25 | -0.13 | -0.25 |
| | Ave. | 0.41 | 0.61 | 0.23 | 0.36 |
| | B | 0.75 | 0.20 | 0.65 | -0.25 |
| | | 0.40 | -0.55 | 0.65 | -0.25 |
| | | 0.05 | -1.05 | 0.55 | -0.10 |
| | | 0.30 | -0.25 | 0.50 | -0.25 |
| | Ave. | 0.38 | -0.41 | 0.59 | -0.21 |
| | Ave. Types x Depths x Soils | 0.39 | 0.10 | 0.41 | 0.08 |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 0.00 | |
| Types (Field Treatment) | 1 | 2.04 | |
| Depths | 1 | 5.25 | |
| Soils | 1 | 1.97 | |
| Types x Depths | 1 | 5.16 | |
| Types x Soils | 1 | 7.07 | |
| Depths x Soils | 1 | 3.99 | |
| Types x Depths x Soils | 1 | 3.67x | 0.68 |
| Error | 55 | 0.46 | |
| Total | 63 | | |

statistically compared with the data in Tables IV, V and VI (where 0.1 gm. of soil was also used for Breton, Winterburn and Edmonton) it does appear that Blue Ridge and Acme soils exhibit no difference in nitrogen fixation from the Breton soil. However, Winterburn and Edmonton soils, especially the latter, appear to fix more nitrogen, under these conditions, than do Blue Ridge and Acme soils. It would seem from the statistical analysis of the data in Table VII that the nitrogen-fixing organism from the Blue Ridge and Acme soils do not vary significantly in their ability to fix nitrogen. The statistically significant interaction types x depths x soils appears to be of no value for interpreting the data.

Table VIII presents data using 0.1 gm. of Duchess and Vauxhall soil as inoculum. Both of these soils are located in irrigated areas; therefore the experiment was designed to determine whether or not irrigation benefits nitrogen-fixation. The analysis of variance carried out on the milligrams of nitrogen fixed per gram of mannite indicates that even for irrigated soils, where the nitrogen-fixing organisms are likely to be shifted about and collected in certain areas, taking duplicate composite field samples appears to be unnecessary. As in Breton soil nitrogen fixation is influenced by depth of soil sample. The 0" - 6" depth is superior to the 6" - 12". This is statistically significant. Irrigation definitely has enhanced nitrogen fixation for Duchess soil. The increase is approximately 12 fold. Although not significantly different the reverse is true of Vauxhall soil. This differential response

TABLE VIII

## Milligrams of Nitrogen Fixed per Gram of Mannite in Soil-Sand Cultures Following Incubation for a Period of 28 Days at 28°C.

One-tenth gm. portions of Vauxhall and Duchess soils were mixed with 50 gm. sand and treated with 10 ml. of a solution containing essential inorganic elements and 0.2 gm. mannite.

| Field Treatment | Duplicate Sample | Field Sample Depth 0" - 6" Vaux. | 0" - 6" Duch. | 6" - 12" Vaux. | 6" - 12" Duch. | Ave. Depths 0" - 6" | Ave. Depths 6" - 12" | Ave. Types x Soils Vaux. | Ave. Types x Soils Duch. |
|---|---|---|---|---|---|---|---|---|---|
| Cultivated Non-Irrigated | A | 0.45 | 0.15 | 1.05 | 1.40 | 2.06 | 1.42 | | |
| | | -0.15 | 0.15 | 0.45 | 0.70 | | | | |
| | | 0.70 | -0.30 | 0.60 | 1.25 | | | | |
| | | 0.10 | -0.45 | -0.10 | 0.15 | | | | |
| | Ave. | 0.28 | -0.11 | 0.50 | 0.88 | | | | |
| | B | 4.85 | 0.60 | 0.35 | -0.10 | | | | |
| | | 0.00 | -0.45 | 0.15 | -0.80 | | | | |
| | | 0.50 | 4.45 | 0.85 | -0.45 | | | | |
| | | 5.80 | 0.55 | 0.40 | -0.40 | | | | |
| | Ave. | 2.79 | 1.29 | 0.44 | -0.44 | | | 1.06 | 0.40 |
| Cultivated Irrigated | A | 1.05 | 7.50 | 0.85 | 7.65 | | | | |
| | | 0.40 | 7.35 | 0.56 | 6.75 | | | | |
| | | 2.30 | 6.45 | 0.25 | 6.25 | | | | |
| | | 0.15 | 7.25 | 1.10 | 0.60 | | | | |
| | Ave. | 0.98 | 7.14 | 0.69 | 5.31 | | | | |
| | B | 2.30 | 7.45 | 0.60 | 8.15 | | | | |
| | | -0.05 | 6.70 | -0.25 | 7.00 | | | | |
| | | 0.35 | 7.30 | 0.25 | 7.45 | | | | |
| | | 0.65 | 7.15 | 0.45 | 7.55 | | | | |
| | Ave. | 0.81 | 7.08 | 0.26 | 7.54 | | | 0.69 | 6.77 |
| Virgin | A | 0.20 | -0.10 | 0.25 | 1.50 | | | | |
| | | 0.35 | 1.15 | 0.70 | 0.45 | | | | |
| | | 5.90 | -0.25 | 0.35 | 1.25 | | | | |
| | | 5.65 | -0.25 | -0.20 | 0.55 | | | | |
| | Ave. | 3.03 | 0.14 | 0.23 | 0.94 | | | | |
| | B | 0.35 | 0.40 | 1.00 | -0.20 | | | | |
| | | 0.30 | 1.15 | 0.60 | -0.20 | | | | |
| | | 0.15 | 1.25 | 0.65 | 0.20 | | | | |
| | | 0.55 | 1.25 | 0.65 | 0.15 | | | | |
| | Ave. | 0.34 | 1.01 | 0.73 | -0.01 | | | 1.09 | 0.52 |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Duplicates | 1 | 0.54 | |
| Types (Field Treatment) | 2 | 94.05** | |
| Depths | 1 | 9.75** | 0.57 |
| Soils | 1 | 64.34 | |
| Types x Depths | 2 | 0.33 | |
| Types x Soils | 2 | 118.73** | 0.99 |
| Depths x Soils | 1 | 1.49 | |
| Types x Depths x Soils | 2 | 0.68 | |
| Error | 83 | 1.97 | |
| Total | 95 | | |

of soils to cultural practices (types) is reflected in types x soils being highly significant. The following explanation may be the probable reason for this differential response. As was established in a later experiment azotobacter were present in cultivated-irrigated Duchess soil but not in cultivated non-irrigated or virgin soil. For the Vauxhall soil azotobacter were present in cultivated non-irrigated and virgin but not in the irrigated soil. Their presence will be discussed later.

(2) Sand Cultures Inoculated With a Soil-Straw Mixture

The main object of these experiments was to determine the possibility that nitrogen-fixing organisms from Edmonton and Winterburn soils, in a natural mixed bacterial flora, could utilize, as energy, the byproducts of different decomposing straws.

The results of these experiments are presented in Tables IX - XIII. Tables IX and X are the results for the first portion of this investigation, which consisted of furnishing the nitrogen-fixing organisms with partly decomposed straws.

Table IX gives the results for six straws (no straw, wheat, oats, barley, alfalfa and flax) receiving water alone and water plus essential inorganic elements. The differences between the amount of nitrogen fixed or lost by the two soils were not statistically significant. This would indicate that under these conditions the nitrogen-fixing organisms in Winterburn and Edmonton soils do not vary significantly in their capabilities of using energy released from these six decomposing straws. In comparing straws it may be seen that alfalfa gave

TABLE IX

## Milligrams of Nitrogen Fixed or Lost in Soil-Sand Cultures

### Following Incubation for 28 days at Room Temperature

Five gm. portions of Winterburn and Edmonton soils, containing 2% straw, were mixed with 50 gm. sand and treated with 10 ml. of various solutions. The soil with the straw was incubated 42 days at optimum moisture before the soil-sand cultures were inoculated.

| Solution Added | No Straw | | Alfalfa | | Flax | | Barley | | Oats | | Wheat | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. |
| Water Only | -0.07 | 0.26 | -0.69 | -0.46 | -0.02 | 0.17 | -0.22 | 0.07 | 0.30 | 0.64 | 0.31 | -0.25 |
| | -0.42 | -0.03 | -0.88 | -0.53 | -0.12 | 0.12 | -0.22 | 0.02 | 0.10 | 0.59 | -0.27 | -0.10 |
| | 0.26 | 0.17 | -0.34 | -0.41 | -0.41 | 0.12 | -0.31 | -0.03 | 0.00 | 0.54 | -0.18 | -0.64 |
| | -0.13 | 0.07 | -0.59 | -0.37 | -0.22 | -0.13 | -0.22 | -0.02 | 0.10 | 0.59 | 0.22 | -0.10 |
| | -0.13 | 0.26 | -0.78 | -0.31 | -1.15 | -0.42 | -0.17 | 0.03 | 0.05 | 0.59 | 0.02 | 0.25 |
| Ave. Soils x Str. x Sol. | -0.10 | 0.15 | -0.66 | -0.42 | -0.38 | -0.05 | 0.23 | 0.02 | 0.11 | 0.59 | 0.02 | -0.17 |
| Water Plus Essential Inorganic Elements | -0.15 | -0.25 | -0.39 | -0.42 | -0.34 | -0.36 | -0.30 | 0.10 | 0.13 | 0.00 | 0.23 | 0.18 |
| | -0.10 | -0.34 | -0.54 | -0.54 | 0.00 | -0.28 | -0.25 | 0.20 | 0.03 | 0.01 | 0.12 | 0.57 |
| | -0.39 | -0.05 | -0.25 | -0.42 | -0.30 | 0.13 | 0.00 | -0.54 | 0.23 | -0.25 | 0.18 | -0.12 |
| | 0.10 | -0.29 | -0.25 | -0.32 | -0.15 | -0.02 | -0.20 | 0.00 | 0.13 | 0.05 | 0.37 | 0.08 |
| | -0.10 | -0.15 | -0.29 | -0.42 | 0.00 | 0.08 | -0.10 | -0.05 | 0.03 | -0.10 | 0.18 | 0.27 |
| Ave. Soils x Str. x Sol. | -0.15 | -0.22 | -0.34 | -0.42 | -0.16 | -0.09 | -0.17 | -0.06 | -0.11 | 0.06 | 0.22 | 0.20 |
| Ave. Str. | -0.16 | | -0.46 | | -0.17 | | 0.01 | | 0.14 | | 0.07 | |

Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Soils | 1 | 0.30x | |
| Straws (Str.) | 5 | 1.08x | 0.28 |
| Solutions (Sol.) | 1 | 0.00 | |
| Soils x Straws | 5 | 0.03 | |
| Soils x Solutions | 1 | 0.50 | |
| Straws x Solutions | 5 | 0.22x | |
| Soils x Straws x Solutions | 5 | 0.12x | 0.33 |
| Error | 96 | 0.04 | |
| Total | 119 | | |

a significant loss in nitrogen when compared to the check and the other straws. This significant loss may be due to alfalfa having a higher protein content, thus greater ammonification has taken place. Oats is the only straw that has given a significant gain in nitrogen fixation by comparison with the check. This would indicate that partly decomposed oat straw releases utilizable nitrogen-fixing energy under these conditions. Effects of inorganic elements in solution are not significant. For soils x straws, soils x solutions, and straws x solutions there was no significant differential response. Although there was a significant differential response of straws in different soils and solutions (soils x straws x solution) no explanation is apparent.

Table X presents the results for the investigation involving three straws (no straw, wheat and alfalfa) and four solutions. Again soils are not statistically different, but unlike the previous experiment straws did not produce statistically significant differences. As might be expected mannite has produced a marked increase in nitrogen-fixation; this difference is highly significant. The interaction soils x solutions is highly significant but this cannot be explained. There was more nitrogen fixed in the case of wheat and alfalfa straws in Winterburn soil than in Edmonton soil. This differential response is reflected in a highly significant interaction for soils x straws. For solutions without mannite alfalfa has produced a significant nitrogen loss when compared to the check; however, in the presence of mannite alfalfa gave a significant increase in nitrogen. Apparently alfalfa in combination with

TABLE X

## Milligrams of Nitrogen Fixed or Lost in Soil-Sand Cultures Following Incubation for 28 Days at Room Temperature

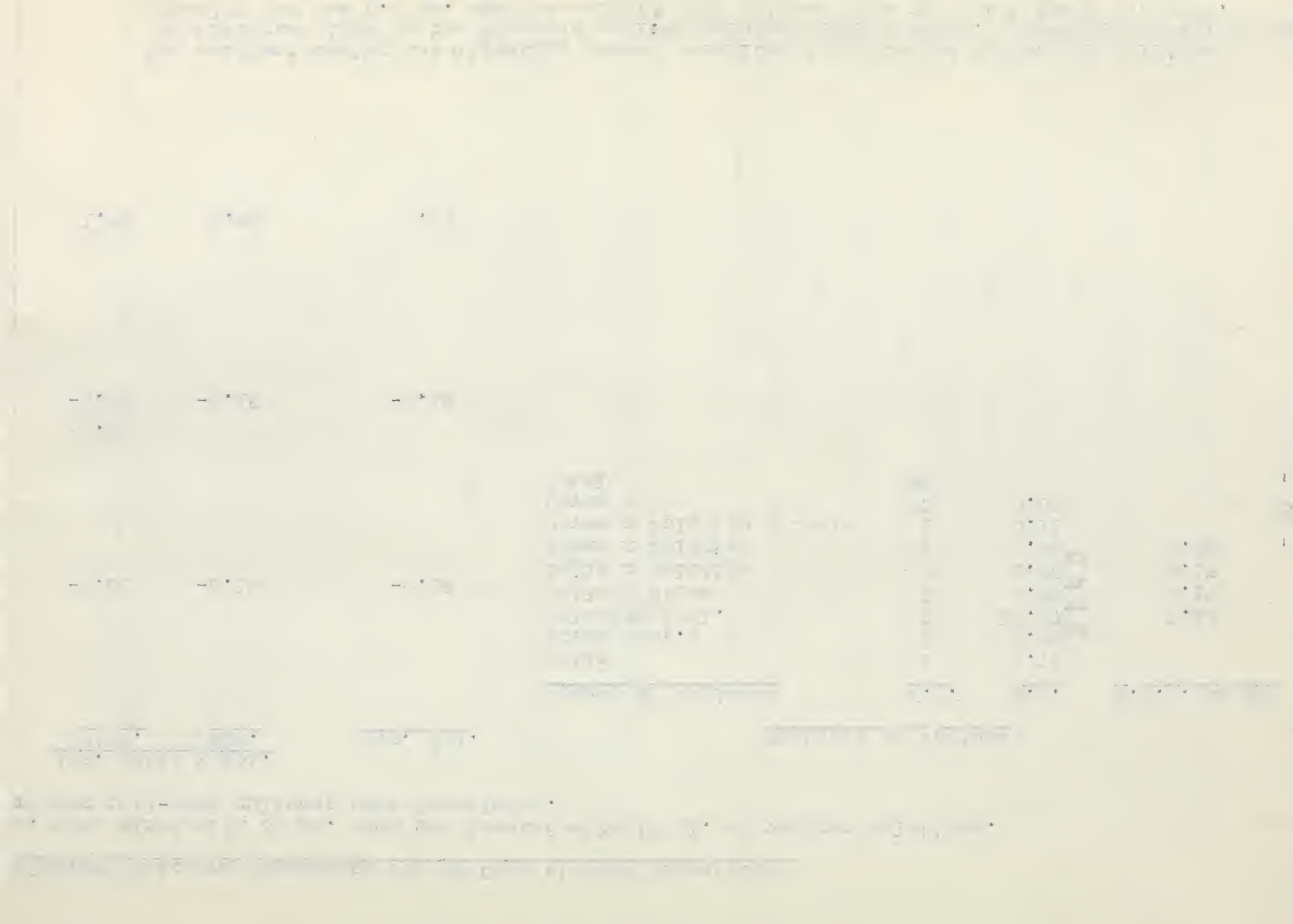
Five gm. portions of Winterburn and Edmonton soils, containing 2% straw, were mixed with 50 gm. sand and treated with 10 ml. of various solutions. The soil with the straw was incubated 42 days at optimum moisture before the soil-sand cultures were inoculated.

| Solution Added | No Straw | | Wheat | | Alfalfa | | Ave. Soils x Sol. | | Ave. Sol. |
|---|---|---|---|---|---|---|---|---|---|
| | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | |
| Water Only | -0.07 | 0.26 | 0.31 | -0.25 | -0.69 | -0.46 | | | |
| | -0.42 | -0.03 | -0.27 | -0.10 | -0.88 | -0.53 | | | |
| | -0.26 | 0.17 | -0.18 | -0.64 | -0.34 | -0.41 | | | |
| | -0.13 | 0.07 | 0.22 | -0.10 | -0.59 | -0.37 | | | |
| | -0.13 | 0.26 | 0.02 | 0.25 | -0.78 | -0.31 | | | |
| Ave. | -0.10 | 0.15 | 0.02 | -0.17 | -0.66 | -0.42 | -0.28 | -0.15 | -0.22 |
| Ave. Str. x Sol. | 0.03 | | -0.08 | | -0.54 | | | | |
| Water Plus Essential Inorganic Elements | -0.15 | -0.25 | 0.23 | 0.18 | -0.39 | -0.42 | | | |
| | -0.10 | -0.34 | 0.12 | 0.57 | -0.54 | -0.52 | | | |
| | -0.39 | -0.05 | 0.18 | -0.12 | -0.25 | -0.42 | | | |
| | 0.10 | -0.29 | 0.37 | 0.08 | -0.25 | -0.32 | | | |
| | -0.10 | -0.15 | 0.18 | 0.27 | -0.29 | -0.42 | | | |
| Ave. | -0.15 | -0.22 | 0.22 | 0.20 | -0.34 | -0.42 | -0.09 | -0.15 | -0.12 |
| Ave. Str. x Sol. | -0.19 | | 0.21 | | -0.38 | | | | |
| Water Plus Essential Inorganic Elements Plus 0.2 Gm. Mannite | 0.96 | 0.31 | 1.62 | 0.69 | 1.65 | 0.83 | | | |
| | 1.16 | 0.26 | 2.40 | 0.88 | 1.45 | 0.81 | | | |
| | 0.96 | 0.41 | 2.30 | 1.32 | 1.70 | 0.72 | | | |
| | 1.18 | 0.46 | 2.10 | 0.49 | 1.60 | 0.23 | | | |
| | 1.25 | 0.36 | 2.15 | 0.83 | 1.50 | 1.25 | | | |
| Ave. | 0.90 | 0.36 | 2.11 | 0.84 | 1.60 | 0.77 | 1.54 | 0.66 | 1.10 |
| Ave. Str. x Sol. | 0.63 | | 1.48 | | 1.19 | | | | |
| Ave. Soils x Str. | 0.22 | 0.10 | 0.78 | 0.40 | 0.20 | -0.02 | | | |
| Water Plus Elements# Plus 0.4 Gm. Mannite | 1.21 | 1.34 | 2.72 | 1.67 | 2.70 | | | | |
| | 0.96 | 1.44 | 2.48 | 1.37 | 2.65 | | | | |
| | 0.96 | 0.90 | 2.72 | 1.57 | 2.40 | | | | |
| | 1.16 | 1.15 | 2.72 | 1.57 | 2.70 | | | | |
| | 1.30 | 0.85 | 2.48 | 1.57 | 2.80 | | | | |
| Ave. | 1.12 | 1.14 | 2.62 | 1.55 | 2.65 | | | | |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Soils | 1 | 1.76 | |
| Straw (Str.) | 2 | 1.75 | |
| Solution (Sol.) | 2 | 15.67** | 0.69 |
| Soils x Straw | 2 | 0.27** | 0.17 |
| Soils x Solution | 2 | 2.05** | 0.17 |
| Straw x Solution | 4 | 0.94** | 0.20 |
| Straw x Solution x Soils | 4 | 0.11 | |
| Error | 73 | 0.05 | |
| Total | 90 | | |

#An accident during the digesting process resulted in distorted values for milligrams of nitrogen fixed in the Edmonton soil containing alfalfa straw. Consequently all of the results for the 0.4 gm. mannite solution were omitted from the statistical analysis.



mannite results in more nitrogen fixed than mannite alone.

Tables XI - XIII present the data for the second portion of this investigation. Here fresh soil-straw mixtures have been added to sand cultures. Table XI gives the data for water alone and water plus essential inorganic elements added to six straws (no straw, alfalfa, barley, wheat, oats and flax). It is of interest to note that effects of undecomposed straws are not statistically significant, whereas effects of partly decomposed straws (Table IX) straws were significant. Thus one would conclude that various partly decomposed straws have different stimulatory effects, but fresh straws do not exhibit such differences. As in Table IX effects of solutions are statistically significant. Adding alfalfa, flax and barley resulted in a nitrogen loss in both soils, however, and the losses in Edmonton soil were significantly lower than the losses in Winterburn soil. This would suggest: (1) Edmonton soil contains more proteolytic bacteria, thus more ammonification has taken place, (2) or if ammonification were the same in both soils, the larger inherent nitrogen content of Edmonton soil has retarded atmospheric nitrogen fixation. Oats again appears to enhance nitrogen-fixation, Winterburn soil being significantly better than Edmonton. In both solutions there is a marked increase in nitrogen fixed for oats when compared to the check; this difference is highly significant.

Table XII gives the data for four solutions added to three straws (no straw, wheat and alfalfa). Both mannite solutions resulted in highly significant increases in nitrogen

TABLE XI

## Milligrams of Nitrogen Fixed or Lost in Soil-Sand Cultures Following Incubation for 28 days at Room Temperature

Five gm. portions of Winterburn and Edmonton soils, containing 2% of straw, were mixed with 50 gm. sand and treated with 10 ml. of various solutions. There was no previous incubation of straw with the soil.

| Solution Added | No Straw | | Alfalfa | | Flax | | Barley | | Oats | | Wheat | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. |
| Water Only | -0.17 | -0.07 | -0.28 | -0.61 | -0.25 | -0.50 | 0.01 | 0.08 | 0.89 | -0.87 | -0.11 | -0.10 |
| | -0.32 | -0.47 | -0.27 | -0.14 | -0.55 | -0.80 | 0.21 | 0.48 | 0.57 | -0.12 | -0.47 | -0.10 |
| | -0.22 | -0.22 | -0.35 | -0.33 | -0.05 | -0.75 | 0.02 | 0.03 | -0.15 | -0.07 | -0.19 | 0.10 |
| | -0.37 | -0.05 | -0.58 | -0.38 | 0.05 | -0.45 | 0.29 | -0.32 | 0.49 | 0.33 | -0.19 | 0.10 |
| | -0.37 | -0.27 | -0.42 | -0.37 | -0.30 | -0.75 | 0.16 | 0.18 | 0.36 | 0.18 | -0.21 | -0.10 |
| Ave. | -0.29 | -0.22 | -0.38 | -0.37 | -0.22 | -0.65 | 0.14 | 0.09 | 0.43 | 0.05 | -0.23 | -0.02 |
| Ave. Str. x Sol. | -0.26 | | -0.38 | | -0.44 | | 0.12 | | 0.24 | | -0.13 | |
| Water Plus Essential Inorganic Elements | -0.28 | -0.38 | 0.13 | -0.55 | -0.28 | -0.37 | -0.13 | -0.05 | -0.18 | -0.15 | -0.18 | -0.03 |
| | -0.38 | -0.13 | -0.37 | -0.61 | -0.08 | -0.67 | -0.33 | -0.40 | 0.07 | 0.30 | -0.35 | 0.12 |
| | -0.28 | -0.18 | -0.02 | -0.95 | -0.58 | -0.87 | 0.19 | -1.10 | -0.13 | 0.15 | -0.05 | 0.17 |
| | -0.20 | -0.23 | -0.37 | -0.35 | -0.31 | -0.42 | -0.21 | -1.00 | 0.27 | -0.20 | -0.10 | -0.48 |
| | -0.38 | -0.18 | -0.12 | -0.25 | -0.18 | -0.57 | -0.12 | -0.20 | -0.13 | -0.25 | 0.00 | -0.08 |
| Ave. | -0.30 | -0.22 | -0.15 | -0.54 | -0.29 | -0.58 | -0.12 | -0.55 | -0.02 | -0.03 | -0.14 | -0.06 |
| Ave. Str. x Sol. | -0.26 | | -0.35 | | -0.44 | | -0.34 | | -0.03 | | -0.10 | |
| Ave. Soil x Str. | -0.30 | -0.22 | -0.27 | -0.46 | -0.26 | -0.62 | 0.01 | -0.23 | 0.21 | 0.01 | -0.19 | -0.04 |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Soils | 1 | 0.48 | |
| Straws (Str.) | 5 | 0.78 | |
| Solutions (Sol.) | 1 | 0.37$^{xx}$ | |
| Soils x Straws | 5 | 0.19$^{xx}$ | 0.20 |
| Soils x Solutions | 1 | 0.04 | |
| Straws x Solutions | 5 | 0.20$^{xx}$ | 0.20 |
| Solutions x Soils x Straws | 5 | 0.11 | |
| Error | 96 | 0.05 | |
| Total | 119 | | |

TABLE XII

## Milligrams of Nitrogen Fixed or Lost in Soil-Sand Cultures Following Incubation for 28 days at Room Temperature

Five gm. portions of Winterburn soils, containing 2% of straw, were mixed with 50 gm. sand and treated with 10 ml. of various solutions. There was no previous incubation of straw with the soil.

| Solution Added | No Straw | | Wheat | | Alfalfa | | Ave. Soils x Sol. | | Ave. Sol. |
|---|---|---|---|---|---|---|---|---|---|
| | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | Wint. | Edm. | |
| Water Only | -0.17 | -0.07 | -0.11 | -0.10 | -0.28 | -0.61 | | | |
| | -0.32 | -0.47 | -0.47 | -0.10 | -0.27 | -0.14 | | | |
| | -0.22 | -0.22 | -0.19 | 0.10 | -0.35 | -0.33 | | | |
| | -0.37 | -0.05 | -0.19 | 0.10 | -0.58 | -0.38 | | | |
| | -0.37 | -0.27 | -0.21 | -0.10 | -0.42 | -0.37 | | | |
| Ave. | -0.29 | -0.22 | -0.23 | -0.02 | -0.38 | -0.37 | -0.30 | -0.20 | -0.25 |
| Ave. Str. x Sol. | -0.26 | | -0.13 | | -0.38 | | | | |
| Water Plus Essential Inorganic Elements | -0.28 | -0.38 | -0.18 | -0.03 | -0.13 | -0.55 | | | |
| | -0.38 | -0.13 | -0.35 | 0.12 | -0.37 | -0.61 | | | |
| | -0.38 | -0.18 | -0.05 | 0.17 | -0.02 | -0.95 | | | |
| | -0.28 | -0.23 | -0.10 | -0.48 | -0.37 | -0.35 | | | |
| | -0.20 | -0.18 | 0.00 | -0.08 | -0.12 | -0.25 | | | |
| Ave. | -0.30 | -0.22 | -0.14 | -0.06 | -0.15 | -0.54 | -0.20 | -0.27 | -0.24 |
| Ave. Str. x Sol. | -0.26 | | -0.10 | | -0.35 | | | | |
| Water Plus Essential Inorganic Elements Plus 0.2 Gm. Mannite | 1.58 | 1.10 | 1.04 | 0.37 | 1.68 | 0.47 | | | |
| | 1.30 | 1.10 | 0.89 | 1.27 | 2.58 | 0.77 | | | |
| | 1.20 | 1.20 | 1.19 | 0.72 | 1.68 | 1.06 | | | |
| | 0.78 | 0.75 | 1.15 | 0.72 | 1.33 | 0.26 | | | |
| | 1.22 | 1.20 | 1.09 | 0.47 | 1.78 | 0.40 | | | |
| Ave. | 1.22 | 1.07 | 1.07 | 0.71 | 1.81 | 0.59 | 1.37 | 0.79 | 1.08 |
| Ave. Str. x Sol. | 1.15 | | 0.89 | | 1.20 | | | | |
| Water Plus Essential Inorganic Elements Plus 0.4 Gm. Mannite | 0.59 | 1.34 | 0.81 | 1.28 | 1.48 | 0.60 | | | |
| | 1.68 | 2.69 | 1.19 | 1.08 | 1.43 | 1.65 | | | |
| | 0.83 | 1.19 | 1.19 | 1.68 | 1.63 | 1.45 | | | |
| | 1.10 | 1.24 | 2.61 | 1.23 | 1.63 | 0.80 | | | |
| | 0.66 | 0.89 | 0.71 | 1.08 | 1.85 | 1.35 | | | |
| Ave. | 0.97 | 1.47 | 1.30 | 1.27 | 1.60 | 1.17 | 1.29 | 1.30 | 1.30 |
| Ave. Str. x Sol. | 1.22 | | 1.29 | | 1.39 | | | | |
| Ave. Soils x Str. | 0.40 | 0.53 | 0.50 | 0.48 | 0.72 | 0.21 | | | |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Soils | 1 | 0.55 | |
| Straws (Str.) | 2 | 0.01 | |
| Solutions (Sol.) | 3 | 20.73$^{xx}$ | 0.24 |
| Soils x Straws | 2 | 1.10$^{x}$ | 0.20 |
| Soils x Solutions | 3 | 0.69$^{x}$ | 0.23 |
| Straws x Solutions | 6 | 0.22 | 0.28 |
| Straws x Solutions x Soils | 6 | 0.16 | |
| Error | 96 | 0.10 | |
| Total | 119 | | |

fixation. The difference between these two were negligible, which indicates that 0.2 gm. of mannite supplies about as much energy for nitrogen fixation as 0.4 gm. mannite. Water alone and water plus essential inorganic elements gave significant losses in nitrogen. This would definitely mean that straws without mannite supplied at the indicated rate do not supply available nitrogen fixing energy, but on the contrary seem to create conditions that promote ammonification or loss of nitrogen. Alfalfa straw in conbination with Winterburn soil is significantly better than in combination with Edmonton soil. For the Winterburn soil there was no significant difference between the 0.2 gm. and 0.4 gm. mannite solution. However for the Edmonton soil the 0.4 gm. mannite was significantly better than the 0.2 gm. mannite. This differential response may mean: (1) the nitrogen-fixing organisms from Winterburn soil utilize energy more efficiently than those from Edmonton soil, (2) or the other microorganisms in Edmonton soil are more competitive than those in Winterburn soil, (3) ammonification may have been greater in Edmonton soil, thus it required more nitrogen fixing energy to compensate for the losses. The interaction straw x solution is statistically significant, but cannot be interpreted without further study.

Table XIII presents the results for a freshly prepared Winterburn soil-straw mixture containing 33 percent straw and receiving four solutions. Since the amount of straw, used for this experiment, was very large in comparison to the other experiments, the quantity of mannite was doubled. Therefore,

TABLE XIII

## Milligrams of Nitrogen Fixed or Lost in Soil-Sand Cultures Following Incubation for 28 days at Room Temperature

Seven and one-half gm. portions of Winterburn soil, containing 33% of straw were mixed with 45 gm. sand and treated with 10 ml. of various solutions. There was no previous incubation of straw with the soil.

| Solution Added | No Straw | Alfalfa (1) | Flax | Barley | Oats | Wheat |
|---|---|---|---|---|---|---|
| Water Only | -0.04 | -33.20 | -5.07 | -0.05 | -0.77 | 0.62 |
| | -0.03 | -35.76 | -5.19 | 3.02 | -0.36 | 0.77 |
| | 0.08 | -35.45 | -5.96 | 2.04 | -0.11 | 0.43 |
| | 0.05 | -36.51 | -4.20 | 0.31 | -1.16 | 0.87 |
| | -0.51 | -36.24 | -4.99 | 0.90 | 0.04 | 0.85 |
| Ave. Str. x Sol. | -0.09 | -35.43 | -5.08 | 1.24 | -0.47 | 0.71 |
| Water Plus Essential Inorganic Elements | 0.23 | -32.72 | -0.58 | 2.18 | -1.24 | 3.90 |
| | 0.02 | -35.58 | -1.26 | 1.07 | -2.91 | 1.78 |
| | -0.10 | -34.22 | -0.28 | 0.57 | -1.05 | 0.20 |
| | 0.08 | -33.82 | -0.58 | -0.18 | -1.72 | 2.74 |
| | 0.08 | -33.29 | -0.48 | 1.97 | -1.64 | 5.09 |
| Ave. Str. x Sol. | 0.05 | -33.93 | -0.64 | 1.12 | -1.71 | 2.74 |
| Water Plus Essential Inorganic Elements Plus 0.4 Gm. Mannite | 2.56 | -33.33 | -0.02 | 0.47 | 1.91 | 0.32 |
| | 2.54 | -35.03 | -0.03 | 0.77 | 1.63 | 0.38 |
| | 2.63 | -35.46 | -2.67 | 0.63 | 0.16 | -0.01 |
| | 2.29 | -36.58 | 0.02 | 2.36 | 0.43 | 0.38 |
| | 2.33 | -33.72 | -0.08 | 0.97 | 1.53 | 0.38 |
| Ave. Str. x Sol. | 2.47 | -34.82 | -0.56 | 1.04 | 1.13 | 0.29 |
| Water Plus Essential Inorganic Elements Plus 0.8 Gm. Mannite | 1.33 | -33.88 | 0.22 | -0.14 | 0.84 | 3.56 |
| | 5.01 | -35.73 | -0.51 | 1.21 | 0.63 | 3.80 |
| | 1.31 | -36.03 | 0.52 | -0.63 | 0.68 | 3.65 |
| | 4.71 | -37.20 | -0.28 | 0.16 | 1.03 | 3.71 |
| | 2.67 | -35.71 | 0.07 | 0.66 | 0.88 | 3.51 |
| Ave. Str. x Sol. | 3.01 | -35.81 | 0.00 | 0.25 | 0.81 | 3.65 |
| Ave. Str. | 1.36 | | -1.57 | 0.91 | -0.06 | 1.85 |

### Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Straws (Str.) | 4 | 36.56x | 2.18 |
| Solutions (Sol.) | 3 | 23.34xx | 1.05 |
| Straws x Solutions | 12 | 9.96 | |
| Error | 80 | 0.70 | |
| Total | 99 | | |

(1) Due to the large loss of nitrogen, the alfalfa wasn't included in the analysis.

instead of the usual 0.2 gm. and 0.4 gm. amounts, the two solutions now contained 0.4 gm. and 0.8 gm. The addition of alfalfa has resulted in a tremendous loss of nitrogen. Of the straws involved in the statistical analysis of these data only flax has given a significant loss in nitrogen. Barley, oats and wheat, when compared to the check, did not result in any significant change in the amount of nitrogen fixed. This would suggest that barley, oats and wheat added to sand cultures at the rates indicated do not stimulate nitrogen fixation. For flax and the check the addition of essential inorganic elements and the further addition of mannite produced a linear response with respect to nitrogen fixation. Although the trend was linear in the case of barley it was in the opposite direction. The response for oats, wheat and barley was irregular. This difference in response of the straws to the different solutions was statistically significant. The reason for this differential response is not apparent. This variation in response is further emphasized by the fact that differences between different solutions are not significantly different as a whole.

(3) Soil Cultures Containing Straw

Table XIV presents the results using Winterburn fine sandy loam as a soil culture. The percentage nitrogen obtained for each replicate, after 35-day incubation period, is reported in two ways. The first column reports the actual percentage nitrogen obtained. The second column reports percentage nitrogen after corrections for loss of organic matter had been applied. Statistical analysis of the data indicates

TABLE XIV

Percentage Nitrogen in Soil Cultures Before and After 35 Days I

Five gm. of straw were added to 100 gm. of Winterburn so

| Solution Added | No Straw | | | Alfalfa | | | Flax | | | Barley | | | Oats | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Water Only | Before Incub. | After | Incub.# | Before Incub. | After | Incub.# | Before Incub. | After | Incub.# | Before Incub. | After | Incub.# | Before Incub. | After |
| | 0.238 | 0.223 | 0.222 | 0.342 | 0.321 | 0.315 | 0.265 | 0.276 | 0.273 | 0.272 | 0.290 | 0.284 | 0.294 | 0.297 |
| | 0.236 | 0.219 | 0.218 | 0.348 | 0.319 | 0.314 | 0.259 | 0.279 | 0.275 | 0.267 | 0.284 | 0.279 | 0.299 | 0.287 |
| | 0.240 | 0.217 | 0.216 | 0.354 | 0.306 | 0.301 | 0.256 | 0.266 | 0.263 | 0.268 | 0.295 | 0.289 | 0.293 | 0.304 |
| | 0.240 | 0.223 | 0.222 | 0.344 | 0.320 | 0.314 | 0.258 | 0.263 | 0.260 | 0.275 | 0.306 | 0.300 | 0.284 | 0.298 |
| | 0.239 | 0.222 | 0.222 | 0.354 | 0.321 | 0.316 | 0.256 | 0.269 | 0.266 | 0.265 | 0.290 | 0.284 | 0.279 | 0.301 |
| Str. x Sol. x Incub. | 0.239 | 0.221 | 0.220 | 0.348 | 0.317 | 0.312 | 0.259 | 0.271 | 0.267 | 0.269 | 0.293 | 0.287 | 0.290 | 0.297 |
| Water Plus Essential Inorganic Elements | 0.259 | 0.230 | 0.229# | 0.361 | 0.302 | 0.295# | 0.248 | 0.262 | 0.258# | 0.279 | 0.291 | 0.285# | 0.285 | 0.298 |
| | 0.250 | 0.231 | 0.231 | 0.359 | 0.304 | 0.297 | 0.253 | 0.265 | 0.262 | 0.280 | 0.290 | 0.285 | 0.273 | 0.300 |
| | 0.256 | 0.234 | 0.234 | 0.355 | 0.307 | 0.300 | 0.255 | 0.259 | 0.255 | 0.279 | 0.290 | 0.285 | 0.277 | 0.294 |
| | 0.250 | 0.235 | 0.234 | 0.343 | 0.318 | 0.310 | 0.252 | 0.260 | 0.257 | 0.274 | 0.291 | 0.286 | 0.291 | 0.291 |
| | 0.254 | 0.238 | 0.237 | 0.350 | 0.296 | 0.289 | 0.253 | 0.262 | 0.259 | 0.280 | 0.292 | 0.286 | 0.296 | 0.277 |
| Str. x Sol. x Incub. | 0.254 | 0.234 | 0.233 | 0.354 | 0.305 | 0.298 | 0.252 | 0.262 | 0.258 | 0.278 | 0.291 | 0.285 | 0.284 | 0.292 |
| Water Plus Essential Inorganic Elements Plus 0.8 Gm. Mannite | 0.231 | 0.238 | 0.236# | 0.348 | 0.333 | 0.325# | 0.261 | 0.262 | 0.258# | 0.271 | 0.293 | 0.286# | 0.301 | 0.292 |
| | 0.236 | 0.238 | 0.236 | 0.346 | 0.318 | 0.311 | 0.255 | 0.256 | 0.252 | 0.288 | 0.300 | 0.293 | 0.299 | 0.286 |
| | 0.245 | 0.238 | 0.237 | 0.359 | 0.314 | 0.307 | 0.251 | 0.263 | 0.258 | 0.290 | 0.292 | 0.286 | 0.302 | 0.264 |
| | 0.231 | 0.236 | 0.234 | 0.348 | 0.318 | 0.311 | 0.251 | 0.259 | 0.255 | 0.285 | 0.300 | 0.293 | 0.308 | 0.305 |
| | 0.236 | 0.236 | 0.234 | 0.343 | 0.316 | 0.309 | 0.251 | 0.265 | 0.260 | 0.286 | 0.292 | 0.286 | 0.305 | 0.276 |
| Str. x Sol. x Incub. | 0.236 | 0.237 | 0.235 | 0.349 | 0.320 | 0.313 | 0.254 | 0.261 | 0.257 | 0.284 | 0.295 | 0.289 | 0.303 | 0.285 |
| Water Plus Essential Inorganic Elements Plus 1.60 Gm. Mannite | 0.230 | 0.251 | 0.249# | 0.364 | 0.330 | 0.324# | 0.257 | 0.249 | 0.245# | 0.260 | 0.305 | 0.298# | 0.293 | 0.297 |
| | 0.228 | 0.253 | 0.250 | 0.371 | 0.329 | 0.320 | 0.253 | 0.265 | 0.261 | 0.278 | 0.305 | 0.297 | 0.289 | 0.312 |
| | 0.240 | 0.238 | 0.235 | 0.368 | 0.327 | 0.319 | 0.249 | 0.267 | 0.263 | 0.277 | 0.305 | 0.298 | 0.282 | 0.310 |
| | 0.241 | 0.241 | 0.240 | 0.351 | 0.332 | 0.323 | 0.244 | 0.266 | 0.261 | 0.273 | 0.308 | 0.301 | 0.278 | 0.318 |
| | 0.217 | 0.242 | 0.240 | 0.346 | 0.329 | 0.320 | 0.243 | 0.267 | 0.262 | 0.269 | 0.300 | 0.292 | 0.277 | 0.318 |
| Str. x Sol. x Incub. | 0.231 | 0.245 | 0.243 | 0.360 | 0.329 | 0.321 | 0.249 | 0.263 | 0.258 | 0.271 | 0.305 | 0.297 | 0.284 | 0.311 |
| Str. x Incub. | 0.240 | 0.234 | 0.233 | 0.353 | 0.318 | 0.311 | 0.254 | 0.264 | 0.260 | 0.276 | 0.296 | 0.290 | 0.290 | 0.296 |
| Str. | | 0.236 | | | 0.327 | | | 0.259 | | | 0.287 | | | 0.292 |

and After 35 Days Incubation in 300 ml. Erlenmeyers at Room Temperature

gm. of Winterburn soil and treated with various solutions.

| Oats | | | Wheat | | | Sol. x Incubation | | |
|---|---|---|---|---|---|---|---|---|
| Before Incub. | After | Incub.# | Before Incub. | After | Incub.# | Before Incub. | After | Incub.# |
| 0.294 | 0.297 | 0.292 | 0.271 | 0.260 | 0.257 | | | |
| 0.299 | 0.287 | 0.282 | 0.265 | 0.261 | 0.258 | | | |
| 0.293 | 0.304 | 0.298 | 0.264 | 0.272 | 0.269 | | | |
| 0.284 | 0.298 | 0.293 | 0.247 | 0.259 | 0.256 | | | |
| 0.279 | 0.301 | 0.295 | 0.262 | 0.270 | 0.267 | | | |
| 0.290 | 0.297 | 0.292 | 0.262 | 0.264 | 0.261 | 0.278 | 0.277 | 0.273# |
| 0.285 | 0.298 | 0.294# | 0.256 | 0.266 | 0.263# | | | |
| 0.273 | 0.300 | 0.295 | 0.251 | 0.267 | 0.264 | | | |
| 0.277 | 0.294 | 0.289 | 0.249 | 0.273 | 0.270 | | | |
| 0.291 | 0.291 | 0.287 | 0.258 | 0.267 | 0.263 | | | |
| 0.296 | 0.277 | 0.273 | 0.263 | 0.268 | 0.265 | | | |
| 0.284 | 0.292 | 0.288 | 0.255 | 0.268 | 0.265 | 0.280 | 0.275 | 0.271# |
| 0.301 | 0.292 | 0.286# | 0.267 | 0.272 | 0.268# | | | |
| 0.299 | 0.286 | 0.279 | 0.270 | 0.272 | 0.267 | | | |
| 0.302 | 0.264 | 0.258 | 0.265 | 0.293 | 0.289 | | | |
| 0.308 | 0.305 | 0.298 | 0.271 | 0.276 | 0.271 | | | |
| 0.305 | 0.276 | 0.269 | 0.267 | 0.268 | 0.264 | | | |
| 0.303 | 0.285 | 0.278 | 0.268 | 0.276 | 0.272 | 0.282 | 0.279 | 0.274# |
| 0.293 | 0.297 | 0.290# | 0.254 | 0.269 | 0.264# | | | |
| 0.289 | 0.312 | 0.304 | 0.245 | 0.268 | 0.263 | | | |
| 0.282 | 0.310 | 0.303 | 0.250 | 0.274 | 0.269 | | | |
| 0.278 | 0.318 | 0.310 | 0.260 | 0.285 | 0.280 | | | |
| 0.277 | 0.318 | 0.310 | 0.239 | 0.294 | 0.289 | | | |
| 0.284 | 0.311 | 0.303 | 0.250 | 0.278 | 0.273 | 0.274 | 0.288 | 0.283# |
| 0.290 | 0.296<br>0.292 | 0.290 | 0.259 | 0.272<br>0.266 | 0.268 | | | |

# Loss of O.M. accounted for.

Analysis of Variance

| Source of Variance | D. F. | M.S. | L.S.D. |
|---|---|---|---|
| Straws (Str.) | 5 | 0.06004$^{xx}$ | 0.006 |
| Solution (Sol.) | 3 | 0.00077 | |
| Incubation (Incub.) | 2 | 0.00070 | |
| Straw x Solution | 15 | 0.00027 | |
| Straw x Incubation | 10 | 0.00267$^{xx}$ | 0.009 |
| Solution x Incubation | 6 | 0.00070$^{xx}$ | 0.008 |
| Straw x Solution x Incubation | 30 | 0.00021$^{x}$ | 0.009 |
| Error | 288 | 0.00005 | |
| Total | 359 | | |

that straws are significantly different. This difference is attributable to the inherent nitrogen content of the straws. Apparently for the straws as a whole, nutrient solutions and incubations did not benefit nitrogen fixation. Comparing the corrected with the non-corrected results there was no instance in which the difference was statistically significant. For flax receiving water only and water plus essential inorganic elements, and for barley receiving water plus essential inorganic elements, and water plus essential inorganic elements plus 0.8 gm. mannite the uncorrected value shows a significant nitrogen increase for the 35 day incubation period, whereas the corrected value shows no significant difference. For all straws receiving water plus essential inorganic elements and water plus essential inorganic elements plus 0.8 gm. mannite, the uncorrected value shows no significant loss in nitrogen while the corrected value shows a significant loss in nitrogen. These last two statements would imply that further experimentation and study is required to ascertain the necessity of correcting for the loss of organic matter. However, since it is a well known fact that organic matter is lost during the decomposition of straw, any significant differences referred to here will be based on the corrected values. Alfalfa has given a significant nitrogen loss regardless of the solution applied. Flax, barley, oats and wheat have given a significant nitrogen increase when receiving water only and water plus essential inorganic elements. From a practical standpoint it is important to note that all of these straws except alfalfa have produced significant increases in nitrogen by comparison with the check soil to which no

straw was added. However, with the addition of 0.8 gm. of mannite, flax, barley, oats and wheat have not produced a significant nitrogen increase, and where 1.6 gm. of mannite was used only barley and wheat gave significant increases in nitrogen. Hence it would appear that under soil conditions, straw in combination with mannite is not superior to mannite alone as a rule.

Table XV presents the results for Duchess irrigated and non-irrigated soil. Three straw treatments were added (no straw, wheat and barley). The solutions added were water alone, and water plus essential inorganic elements, which were added until 60 percent of the water holding capacity (air-dry basis) was reached. The portion of these soil-straw mixtures to be placed under anaerobic conditions then received distilled water until the moisture content was 105 percent of the water holding capacity (air-dry basis). All reported nitrogen analyses of replicates taken after the 35-day incubation period are reported as the corrected values. The difference between straws and the difference between soils is highly significant. As in Table XIV this difference is attributable to their inherent nitrogen content. Under both anaerobic and aerobic conditions, for straws as a whole, the 35-day incubation period has produced a significant loss of nitrogen. The amount of nitrogen lost under anaerobic conditions is significantly more than that lost under aerobic conditions. This would indicate that more ammonification takes place under field conditions if the soil is water logged assuming that the nitrogen is lost in the form of

TABLE XV. Percentage Nitrogen in Soil Cultures Before and After 35 Days Incubation in 300 ml. Erlenmyers at Room Temperature

Five gm. of straw were added to 100 gm. of Duchess cultivated non-irrigated and cultivated irrigated soil.

| Field Treatment | Straw Added | Before Incubation | | After Incubation (Aer.) | | After Incub. (Anaer.) | |
|---|---|---|---|---|---|---|---|
| | | $H_2O$ Alone | $H_2O$ + Essent. Inorg.Elements | $H_2O$ Alone | $H_2O$ + Essent. Inorg.Elements | $H_2O$ Alone | $H_2O$ + Essent. Inorg.Elements |
| Cultivated Irrigated | No Straw | 0.218 | 0.218 | 0.209 | 0.207 | 0.191 | 0.199 |
| | | 0.191 | 0.209 | 0.212 | 0.214 | 0.205 | 0.199 |
| | | 0.217 | 0.213 | 0.214 | 0.211 | 0.205 | 0.203 |
| | | 0.209 | 0.214 | 0.207 | 0.213 | 0.206 | 0.202 |
| | | 0.220 | 0.218 | 0.207 | 0.214 | 0.204 | 0.203 |
| Ave. Soils x Str. x Incub. x Sol. | | 0.211 | 0.212 | 0.209 | 0.212 | 0.202 | 0.201 |
| | Wheat | 0.233 | 0.229 | 0.221 | 0.232 | 0.225 | 0.227 |
| | | 0.231 | 0.232 | 0.209 | 0.233 | 0.226 | 0.226 |
| | | 0.235 | 0.230 | 0.222 | 0.234 | 0.222 | 0.231 |
| | | 0.231 | 0.232 | 0.223 | 0.242 | 0.224 | 0.226 |
| | | 0.231 | 0.231 | 0.218 | 0.230 | 0.220 | 0.228 |
| Ave. Soils x Str. x Incub. x Sol. | | 0.230 | 0.231 | 0.219 | 0.234 | 0.203 | 0.228 |
| | Barley | 0.243 | 0.247 | 0.251 | 0.248 | 0.232 | 0.240 |
| | | 0.251 | 0.249 | 0.234 | 0.246 | 0.236 | 0.235 |
| | | 0.252 | 0.260 | 0.240 | 0.249 | 0.232 | 0.235 |
| | | 01250 | 0.251 | 0.249 | 0.237 | 0.230 | 0.238 |
| | | 0.248 | 0.255 | 0.253 | 0.246 | 0.231 | 0.232 |
| Ave. Soils x Str. x Incub. x Sol. | | 0.249 | 0.252 | 0.245 | 0.245 | 0.232 | 0.236 |
| Cultivated Non-Irrigated | No Straw | 0.233 | 0.243 | 0.242 | 0.247 | 0.235 | 0.242 |
| | | 0.234 | 0.247 | 0.217 | 0.246 | 0.235 | 0.237 |
| | | 0.240 | 0.238 | 0.240 | 0.241 | 0.237 | 0.238 |
| | | 0.239 | 0.244 | 0.237 | 0.247 | 0.235 | 0.241 |
| | | 0.234 | 0.240 | 0.235 | 0.249 | 0.235 | 0.238 |
| Ave. Soils x Str. x Incub. x Sol. | | 0.236 | 0.242 | 0.234 | 0.246 | 0.235 | 0.239 |
| | Wheat | 0.253 | 0.265 | 0.255 | 0.261 | 0.261 | 0.263 |
| | | 0.265 | 0.269 | 0.255 | 0.256 | 0.265 | 0.254 |
| | | 0.268 | 0.265 | 0.264 | 0.263 | 0.255 | 0.256 |
| | | 0.265 | 0.263 | 0.260 | 0.263 | 0.261 | 0.254 |
| | | 0.263 | 0.268 | 0.244 | 0.260 | 0.259 | 0.258 |
| Ave. Soils x Str. x Incub. x Sol. | | 0.263 | 0.266 | 0.256 | 0.261 | 0.260 | 0.257 |
| | Barley | 0.272 | 0.275 | 0.270 | 0.268 | 0.262 | 0.270 |
| | | 0.275 | 0.276 | 0.268 | 0.276 | 0.265 | 0.270 |
| | | 0.277 | 0.275 | 0.275 | 0.279 | 0.265 | 0.266 |
| | | 0.283 | 0.273 | 0.252 | 0.263 | 0.261 | 0.263 |
| | | 0.274 | 0.277 | 0.268 | 0.283 | 0.267 | 0.266 |
| Ave. Soils x Str. x Incub. x Sol. | | 0.276 | 0.275 | 0.267 | 0.274 | 0.264 | 0.267 |

Ave. Solutions

| $H_2O$ Alone | $H_2O$ + Essential Inorganic Elements |
|---|---|
| 0.240 | 0.243 |

Ave. Incubation

| Before Incub. | After Incub. (Aer.) | After Incub. (Anaer.) |
|---|---|---|
| 0.246 | 0.242 | 0.237 |

Analysis of Variance

| Source of Variance | D.F. | M.S. | L.S.D. at 5% |
|---|---|---|---|
| Soils | 1 | 0.04065xx | |
| Straws (Str.) | 2 | 0.01689xx | |
| Incubations (Incub.) | 2 | 0.00110x | 0.003 |
| Solutions (Sol.) | 1 | 0.00060x | 0.003 |
| Soils x Straws | 2 | 0.00011 | |
| Soils x Incubations | 2 | 0.00013 | |
| Soils x Solutions | 1 | 0.00001 | |
| Straws x Incubations | 4 | 0.00014 | |
| Straws x Solutions | 2 | 0.00002 | |
| Incubations x Solutions | 2 | 0.00012 | |
| Soils x Straws x Incubations | 4 | 0.00003 | |
| Soils x Straws x Solutions | 2 | 0.00010 | |
| Soils x Incubations x Solutions | 2 | 0.00001 | |
| Straws x Incubations x Solutions | 4 | 0.00004 | |
| Soils x Straws x Incubations x Solutions | 4 | 0.00006x | 0.008 |
| Error | 144 | 0.00002 | |
| Total | 179 | | |

ammonia. There is a significant difference between water plus essential inorganic elements and water alone. Apparently the essential inorganic elements have benefited nitrogen fixation or they have prevented greater ammonification or loss of nitrogen. If they have benefited nitrogen fixation it is probably due to the beneficial effect of the phosphorus, because the soils from the brown soil zone are known to be low in available phosphorus.

As compared to the check (no straw), water (alone) in combination with barley or wheat and cultivated irrigated soil has given a significant decrease in nitrogen regardless of whether aerobic or anaerobic conditions were used. However, where water plus essential inorganic elements was used no significant decreases occured. Under both conditions water (alone) added to the combination of barley and cultivated non-irrigated soil has given a significant decrease in nitrogen, whereas when added to the combination of wheat and cultivated non-irrigated soil no significant decrease in nitrogen occured. Water plus essential inorganic elements added to barley in combination with cultivated non-irrigated soil did not give a nitrogen decrease, but wheat under aerobic conditions did give a significant nitrogen-decrease. Water plus essential inorganic elements added to wheat in combination with cultivated irrigated soil and under anaerobic conditions is the only combination that gave a significant increase in nitrogen. These changes in trends have caused the interaction soils x straws x incubations x solutions to be significant.

In reviewing the results of Table XIV and XV the analyses of variance indicate that it is possible to measure the nitrogen increases or decreases that occur in soil cultures. Apparently straws low in protein favor nitrogen fixation when added to Winterburn soil. However, in combination with Duchess soil there seems to be a decrease in nitrogen. This difference is hard to explain, because as revealed in a later experiment no azotobacter could be isolated from Winterburn soil, but they were isolated from the Duchess irrigated soil. Because of the fact that Duchess soil is a silt loam, anaerobic conditions may have prevailed below the soil surface even where only 60 percent moisture was present thus preventing azotobacter from fixing nitrogen and if anaerobic nitrogen-fixing organisms were present, they were apparently unable to utilize the byproducts of decomposing straw. Or, on the other hand, more proteolytic organisms may have been present in the Duchess than in the Winterburn soil, thus nitrogen fixation did not compensate for the loss due to ammonification.

(4) Bacteriological Examination

Attempts were made to isolate azotobacter from both depths in all soils used in this experiment. Inocula from all soils when introduced into a nitrogen-free liquid medium formed a pellicle after two to three days of incubation. However only inocula from three soils produced profuse growths, whereas in the case of the other soils only scant growths were observed. The inocula that gave rise to heavy growths in the liquid medium were from Duchess cultivated irrigated, Vauxhall virgin and Vauxhall cultivated non-irrigated. Upon microscopic

view

examination of the pellicles it was found that only these three soils showed the presence of typical azotobacter cells. It was also found that upon plating onto nitrogen-free agar plates that these were the only cultures that produced typical azotobacter colonies. They were found in both soil depths.

Although in all controls (sterile medium and soil, inoculated with a pure culture of azotobacter) only a scant growth of azotobacter was observed, it should still indicate that no adverse conditions were present in any of these cultures. For as Waksman (76) states the absence of contaminants retards azotobacter growth.

Table XVI gives the milligrams of nitrogen fixed per gram of mannite by pure cultures of these isolated azotobacter. Only the azotobacter from the 0" - 6" depths were used. As shown in the Table these azotobacter were able to fix atmospheric nitrogen. However, apparently they show no significant differences in nitrogen fixing ability.

It is to be noted (see Table I) that azotobacter were only isolated from soils that have a pH that falls well within the range of 6.5 - 8.6 given by Fred and Davenport (18) as the optimum for aerobic nitrogen-fixing organisms. The reader may then wonder why azotobacter could not be found in Acme soil and in Duchess cultivated non-irrigated soil where the average pH is about 7.0.

The presence of salts, which may be harmful, in these solonetz-like soils could be the probable answer. In the case of Duchess cultivated irrigated soil, irrigation water may have removed the harmful salts or brought in strains of

TABLE XVI Milligrams of Nitrogen Fixed per Gram of Mannite in Sand Cultures Following Incubation for a Period of 21 Days at 28°C.

One ml. of pure solution cultures of azotobacter that had been isolated from Duchess cultivated irrigated, Vauxhall virgi and Vauxhall cultivated non-irrigated were used to inoculate 50 gm. of sterile sand in 300 ml. erlenmyers. Each flask received 10 ml. of a solution containing essential inorganic elements and 0.2 gm. of mannite.

| Azotobacter from Duchess Cult. Irr. | Azotobacter from Vauxhall Virgin | Azotobacter from Vauxhall Cult. Non-Irr. |
|---|---|---|
| 2.49 | 1.73 | 0.89 |
| 1.93 | 2.28 | 1.59 |
| 2.07 | 2.83 | 1.66 |
| Ave. 2.16 | 2.28 | 1.38 |

Analysis of Variance

| Source of Variance | D. F. | M.S. |
|---|---|---|
| Soils | 2 | 0.72 |
| Error | 6 | 0.43 |
| Total | 8 | |

azotobacter that could grow under these conditions.

The fact that azotobacter were absent from, Breton, Winterburn and Edmonton soil indicates that in sand cultures containing these soils other important nitrogen-fixing organisms are present. If further studies are carried out it would be interesting to determine what organisms these are.

## SUMMARY

(1) A study was made to determine some of the factors influencing the nitrogen-fixing powers of virgin and cultivated soils from the four major soil zones of Alberta.

(2) It was found that single composite soil samples are as reliable as duplicate composite soil samples when determining the nitrogen fixing powers of these soils.

(3) Experiments with sand cultures inoculated with soil or soil-straw mixtures showed that Vauxhall, Duchess, Acme, Edmonton, Winterburn, Breton and Blue Ridge soils vary in their ability to fix nitrogen.

(4) More nitrogen was fixed in sand cultures inoculated with 0.5 gm. than with 0.1 gm. of soil. The less fertile the soil the greater was this difference.

(5) In some cases it was found that virgin soil fixed markedly less nitrogen than the cultivated soil.

(6) The 0" - 6" soil depth generally fixed more nitrogen than the 6" - 12" soil depth.

(7) A 28-day incubation period seems to be as satisfactory as a 49-day period when determining the amount of nitrogen fixed by a given soil.

(8) Breton soil treated with ammonium sulfate and complete fertilizer since 1930 fixed no more nitrogen than the untreated soil.

(9) Irrigation waters apparently have improved nitrogen-fixing power in a solonetz-like Duchess soil.

(10) The nitrogen-fixing organisms from Edmonton soil utilize mannite more efficiently when the mannite is in smaller concentration.

(11) Nitrogen-fixing organisms from Winterburn and Edmonton soil are able, under certain conditions, to utilize energy released from various decomposing straws.

(12) Nitrogen is lost when alfalfa straw, without mannite, is added to sand and soil cultures.

(13) Cereal straws, without mannite, when added to Winterburn cultivated soil aid nitrogen fixation. However, when added to Duchess cultivated and Duchess cultivated non-irrigated soil nitrogen is lost under the conditions of these experiments.

(14) Essential inorganic elements (other than nitrogen) seemed to enhanced nitrogen fixation when added to Duchess soil, but no response was observed when added to other soils.

(15) More nitrogen is lost during the period of incubation when Duchess soil is waterlogged than when it is maintained at an optimum moisture content.

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